METHODS

Association analyses. The Malmö Diet and Cancer Study – Cardiovascular Cohort (MDC-CC) is a prospective, community-based epidemiological cohort of 6,103 residents of Malmö, Sweden, for whom a comprehensive analysis of cardiovascular risk factors has been performed. The 1p13 SNP rs646776 was genotyped as previously described[34]. The ion mobility method of lipoprotein measurement was applied to archived baseline blood samples from these individuals to directly quantify the full spectrum of lipoprotein particles, as previously described[34]. Multivariable linear regression analyses were used to test whether each of the lipid or lipoprotein measures differed according to an increasing copy number of the SNP minor allele, adjusted for age, gender, and diabetes status. SPSS (version 16.0) was used for the analyses.

The Pharmacogenomics and Risk of Cardiovascular Disease (PARC) study is a two-stage genome-wide association (GWA) study[35]. In stage 1, 980 subjects were typed for 317,000 SNPs with the Illumina Human-1 BeadChip. In stage 2, 930 additional subjects were typed for a subset of 13,680 SNPs with the Illumina iSelect platform. All subjects were of self-reported European ancestry. The gradient gel electrophoresis method of lipoprotein measurement was applied to blood samples from these individuals to directly quantify the full spectrum of lipoprotein particles, as previously described[36]. Multivariable linear regression analyses were used to test whether each of the lipid or lipoprotein measures differed according to an increasing copy number of the SNP minor allele. JMP (SAS Institute) was used for the analyses.

Roughly 20,000 individuals of European descent were genotyped on various array platforms, and roughly 9,000 African Americans individuals were genotyped on the ITMAT-Broad-CARe Array (Illumina). Association analyses for LDL-C and meta-analyses were performed as previously described[37, 38].

Genotype-expression analyses. To evaluate whether SNPs serve as eQTLs with putative *cis* regulatory effects on liver and adipose gene expression traits, 782,476 SNPs had been genotyped

and expression levels of 39,280 transcripts profiled in 960 human liver samples, 433 human subcutaneous adipose samples, and 520 human omental adipose samples. Tissue samples were either postmortem or surgical resections from organ donors or elective cases. Methods for tissue collection, RNA and DNA isolation, expression profiling, and DNA genotyping have been previously described[39]. The correlation of rs646776 minor allele count with each of the profiled transcripts was determined using linear regression analysis.

For the replication study in 62 liver samples, de-identified histopathologically normal human liver samples were provided by the University of Minnesota Academic Health Center's Biological Materials Procurement Facility (BioNet; www.bionet.umn.edu). For each sample, 1 μg of DNAase-treated total RNA was converted into cDNA with random hexamers and SuperScript III reverse transcriptase (Invitrogen). cDNA samples were diluted with water and 2 ng of total RNA was used for each quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), performed with TaqMan Gene Expression Assays for *SORT1*, *PSRC1*, *CELSR2*, *TCF7L2*, and *B2M* (beta-2-microglobulin) and associated reagents (Applied Biosystems) according to the manufacturer's protocol. Expression of all assays was measured in technical duplicates and average values of the duplicates were used for the analysis. The *SORT1*, *PSRC1*, *CELSR2*, and *TCF7L2* expression values for each target were normalized by *B2M* expression values (ΔCt method) and were tested for normality of distribution prior to analysis. A pre-developed TaqMan genotyping allelic discrimination assay for SNP rs12740374 was used according to the manufacturer's protocol (Applied Biosystems). A univariate linear regression analysis was used to test the associations between mRNA expression and the SNP coded by the number of major alleles and was performed with SPSS 16.0. Information on age and sex was tested as a covariate but was not included in the final analysis as it was not available for all samples.

Protein extracts from liver tissue samples were prepared by homogenization of ~ 30 mg of tissue with Tissue Lyser (QIAGEN) in RIPA buffer (Invitrogen) in the presence of Complete

cocktail of proteinase inhibitors (Roche). Samples were subjected to immunoblotting and probed with anti-sortilin antibody (AF2934, R&D Systems) or anti-α-tubulin antibody as loading control (ab-7291-100, Abcam).

Luciferase expression constructs. To characterize the intergenic region between *CELSR2* and *PSRC1*, the major (Hap1) and minor (Hap2) haplotypes from two bacterial artificial chromosomes (CTD-2068B15 and RP11-463O24, respectively; Invitrogen) were cloned into the pGL3-Promoter vector (Promega) in both the 5′-to-3′ and 3′-to-5′ orientations just downstream of the stop codon of the firefly luciferase gene. A naturally occurring BamHI site was used to generate constructs with truncations and composites of the two haplotypes. PCR was to generate smaller truncations. The QuikChange Site-Directed Mutagenesis Kit (Stratagene) was used to alter single nucleotides (i.e., SNP alleles). All constructs were verified by DNA sequencing.

Luciferase expression assays. Hep3B cultured human hepatoma cells, BNL CL.2 cultured mouse embryonic liver cells, or NIH 3T3 cultured mouse fibroblast cells were transfected at roughly 50% confluence and maintained in DMEM + 10% FBS. In some experiments, cells were infected with a lentivirus encoding the C/EBPα cDNA or a lentivirus encoding the A-C/EBP[40, 41] (dominant negative C/EBP) cDNA 24 hours prior to transfection. The firefly luciferase constructs were co-transfected with the Renilla luciferase pRL-CMV Vector (Promega) using the FuGENE 6 transfection reagent (Roche) in the ratio 1 μg:100 ng:3 μl mixed with Opti-MEM I Reduced Serum Medium (Invitrogen) for a 100 μl mix, of which 20 μl was used for each well of 24-well plates. Forty-eight hours after transfection, firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol, using untransfected cells to adjust for background activity.

***SORT1* expression assays.** Hep3B cultured human hepatoma cells or SK-HEP-1 cultured human hepatoma cells were seeded at roughly 50% confluence in 24-well plates and infected with a lentivirus encoding the A-C/EBP (dominant negative C/EBP) cDNA or mock-infected; virus was

removed after 24 hours. The cells were maintained in DMEM + 10% FBS. Seventy-two hours after infection, total RNA was isolated with the RNeasy Mini Kit (QIAGEN).

HUES-1 or HUES-9 human embryonic stem cells were seeded at roughly 50% confluence on Geltrex matrix (Invitrogen) and initially maintained on mTeSR1 medium (StemCell Technologies). The cells were then switched to and maintained on EndoMedia (RPMI-B27 medium, supplemented with 100 ng/ml human recombinant activin A, Invitrogen) for seven days to induce differentiation into definitive endoderm. Successful differentiation was confirmed in parallel experiments by monitoring morphological changes and detecting expression of endoderm-specific markers *SOX17* and *GATA4*. C/EBPα was expressed via lentiviral infection during the last two days of differentiation, followed by isolation of total RNA with the RNeasy Mini Kit (QIAGEN).

For each sample, 2 μg of total RNA was converted into cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was performed with TaqMan Gene Expression Assays for *SORT1* and *B2M* and associated reagents (Applied Biosystems) according to the manufacturer's protocol. The *SORT1* expression values for each target were normalized by *B2M* expression values (ΔCt method).

Electrophoretic mobility shift assays (EMSA). Primers with the consensus C/EBPα binding site were previously described[42]: C/EBPα-F, 5′-CTAGGCATATTGCGCAATATGC-3′; C/EBPα-R, 5′-GCATATTGCGCAATATGCCTAG-3′. Primers for rs12740374 were designed based on genomic sequences surrounding the SNP (http://www.ncbi.nlm.nih.gov/projects/SNP/): rs12740374_T-F, 5′-TGCCCTGAGG**T**TGCTCAATCA-3′; rs12740374_T-R, 5′-TGATTGAGCAA**C**CTCAGGGCA-3′; rs12740374_G-F, 5′-TGCCCTGAGG**G**TGCTCAATCA-3′; rs12740374_G-R: 5′-TGATTGAGCA**C**CCTCAGGGCA-3′. All primers were ordered from Invitrogen. Individual primers were labeled with a biotin 3′ end DNA labelling kit (Pierce) according to instructions,

and the efficiency of labeling was tested by a dot-test that confirmed that all the primers were labeled similarly. Corresponding forward and reverse primers were annealed to create 3′ end biotin-labelled double-stranded probes. EMSA reactions were performed with the biotin 3′ end DNA labelling kit (Pierce) according to instructions, with 8 μg of nuclear extract from HepG2 cultured human hepatoma cells per reaction (Active Motif). For competition assays, we used 100-fold excess of unlabeled probe. To test for involvement of CEBP/α in interaction with the probes, we preincubated the HepG2 nuclear extract for 15 min at room temperature with either of two antibodies for CEBP/α (39306, Active Motif; 2295, Cell Signaling). The protein complexes were resolved on 6% DNA retardation gels (Invitrogen) for 1 hour at 100 V, transferred to Biodyne B Nylon Membranes (Pierce), crosslinked, and processed with the Chemiluminescent Nucleic Acid Detection Module (Pierce).

Chromatin immunoprecipitation assays. HUES-1 human embryonic stem cells were seeded at roughly 50% confluence on Geltrex matrix (Invitrogen) and maintained on mTeSR1 medium (StemCell Technologies). The cells were infected with a lentivirus encoding C/EBPα; virus was removed after 24 hours. Seventy-two hours after infection, the cells were harvested and cross-linked with 4% paraformaldehyde at 37°C for 10 minutes followed by quenching with glycine and flash freezing. After thawing, the lysates were sonicated in RIPA buffer 25 times for 10 sec at 4°C. The lysates were precipitated with anti-C/EBPα antibody (2295, Cell Signaling) at 1:40 dilution overnight vs. no antibody. After incubation with Protein G Sepharose beads (GE Healthcare) for two hours at room temperature, serial washes, and elution, DNA was recovered by addition of sodium chloride and incubation overnight at 65°C, followed by treatment with proteinase K and RNase A for two hours at 42°C. DNA was purified with the QIAquick PCR Purification Kit (QIAGEN). The presence of immunoprecipitated DNA sequence around rs12740374 was assayed by quantitative PCR using the primers 5′-CTGAGGTTGCTCAATCAAGCGCTTGATTGAGCAACCTCAG-3′ and 5′-CTGAGGGTGCTCAATCAAGCGCTTGATTGAGCACCCTCAG-3′ and the probe 5′-FAM-

AGCCAGCACTGTGTTTACTCTTCCTC-Iowa Black-3′ (Integrated DNA Technologies). The values for the immunoprecipitated target were normalized by values for the target from 1:30 dilution of input chromatin.

siRNA screening and validation. siRNA design was carried out to identify siRNAs targeting both homologues of the gene *SORT1* from human (symbol *SORT1*) and mouse (symbol *Sort1*). The design used the *SORT1* transcripts NM_002959.4 (human) and NM_019972.2 (mouse) from the NCBI RefSeq collection. siRNA duplexes were designed with 100% identity to both respective *SORT1* genes. To select appropriate candidate target sequences and their corresponding siRNAs, their predicted potentials for interacting with irrelevant targets (off-target potentials) were used as a ranking parameter. siRNAs with low off-target potentials were defined as preferable and assumed to be more specific *in vivo*. To identify potential off-target genes, 19-mer candidate sequences were subjected to a homology search against the human and mouse RefSeq mRNA databases. The following off-target properties for each 19-mer input sequence were extracted for each off-target gene to calculate the off-target score: number of mismatches in nonseed region, number of mismatches in seed region, and number of mismatches in cleavage site region. The twenty-nine siRNAs with best off-target scores were selected for synthesis and screening.

Single-stranded RNAs were produced at Alnylam Pharmaceuticals as previously described[43, 44]. Deprotection and purification of the crude oligoribonucleotides by anion exchange high performance liquid chromatography (HPLC) were carried out according to established procedures. siRNAs were generated by annealing equimolar amounts of complementary sense and antisense strands.

For screening transfection experiments, BNL CL.2 cultured mouse embryonic liver cells were seeded at 4 x 10^4 cells per well in 24-well plates and reverse transfected with the siRNAs using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. For each

sample, 2 μg of total RNA was converted into cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Forty-eight hours after transfection, total RNA was isolated with the RNeasy Mini Kit (QIAGEN). For each sample, 2 μg of total RNA was converted into cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was performed with TaqMan Gene Expression Assays for *Sort1* and 18S rRNA and associated reagents (Applied Biosystems) according to the manufacturer's protocol. The *Sort1* expression values for each target were normalized by 18S rRNA expression values (ΔCt method). A half-maximal inhibitory concentration (IC_{50}) curve was determined for the *Sort1* duplex yielding the greatest degree of knockdown. The sequences for this duplex were: 5′-uGucAGAAuGGucGAGAcudTsdT-3′ and 5′-AGUCUCGACcAUUCUGAcAdTsdT-3′ (2′-OMe modified nucleotides are in lower case, and phosphorothioate linkages are indicated by "s").

A previously validated siRNA duplex targeting the luciferase gene was used[43].

Gene knockdown studies in mouse liver. Lipidoid formulations of siRNAs were prepared as previously described[43]. Mice received either phosphate-buffered saline (PBS) or formulated siRNAs via weekly tail vein injection at dosages of 2.0 mg/kg. At various timepoints (including prior to injection), animals were anesthesized by isofluorane inhalation, and blood was collected by retro-orbital bleed followed by centrifugation to isolate plasma. Mice were sacrificed at five days or at two weeks after four hours of fasting. After sacrifice of the mice, terminal bleeds were collected, and livers and adipose were harvested and snap frozen in liquid nitrogen. Frozen tissue was ground, and tissue lysates were prepared. *Sort1* mRNA levels relative to those of *GAPDH* mRNA were determined in the liver lysates by using the branched-DNA-technology-based QuantiGene Reagent System (Panomics), according to the manufacturer's protocols. Sortilin and actin expression in liver and adipose was determined by immunoblotting (612100, BD Transduction Laboratories; ab20272, Abcam).

5′-RACE was conducted as previously described[44]. In brief, an oligonucleotide adaptor was ligated to total liver RNA, and the ligation mixture was reverse transcribed using the *Sort1*-specific oligonucleotide 5′-tattccaggaggtcctcatctgagtcgtC-3′, followed by cDNA amplification with the oligonucleotides 5′-CGACTGGAGCACGAGGACACTGACATGG-3′ and 5′-Ggattcatcccaccttggcatttgtctc-3′. Nested PCR was performed using the oligonucleotides 5′-GGACACTGACATGGACTGAAGGAGTAG-3′ and 5′-gaagtagccaaagtcacagaggaagtc-3′. PCR products were examined by gel electrophoresis, purified, and subcloned for sequencing.

Sort1–/– mice were generated as previously described[45] and outbred to the C57BL/6 strain. Matched wild-type C57BL/6 mice were used as controls.

All mice were fed ad libitum with regular rodent chow. All procedures used in animal studies were approved by the pertinent Institutional Animal Care and Use Committee and were consistent with local, state, and federal regulations as applicable.

Gene overexpression studies in mouse liver. The murine *Sort1* cDNA (Origene, MR210834) was subcloned into a specialized vector for use by the University of Pennsylvania's Penn Vector Core for production of AAV8 viral particles expressing *Sort1*. Viruses were produced with a chimeric packaging construct in which the AAV2 rep gene was fused with the cap gene of AAV serotype 8[46]. Empty AAV8 viral particles were also provided by the Penn Vector Core.

Mice received either 1 x 10^{12} viral particles of null AAV or 1 x 10^{12} viral particles of AAV encoding *Sort1* in PBS via intraperitoneal injection. At various timepoints (including prior to injection), animals were anesthesized by isofluorane inhalation and blood was collected by retro-orbital bleed followed by centrifugation to isolate plasma. Mice were sacrificed at six weeks upon four hours of fasting. After sacrifice of the mice, terminal bleeds were collected and livers and adipose were harvested and analyzed for protein and gene expression as described above.

Measurement of mouse plasma lipids and lipoproteins. Collected mouse plasma samples were analyzed for lipids by analytical chemistry and fast protein liquid chromatography (FPLC) and for lipoproteins by nuclear magnetic resonance (NMR). Total plasma cholesterol and alanine aminotransferase (ALT) were measured enzymatically on a Cobas Mira autoanalyzer (Roche Diagnostic Systems). Pooled plasma from each experimental group (140 μl) was separated by FPLC gel filtration. Cholesterol and triglyceride plate assays were performed on FPLC fractions using the Infinity cholesterol and triglyceride reagents, respectively. Individual plasma samples were sent for NMR lipoprotein measurement (LipoScience).

VLDL secretion studies. To study hepatic VLDL secretion, mice were prebled by retro-orbital bleeding followed by intraperitoneal injection of 400 μl of 1 mg/g Pluronic F-127 detergent resuspended in PBS. The mice were fasted for four hours prior to injection and through the study. We performed serial retro-orbital bleeds at one, two, and four hours after injection of the detergent. Plasma samples were individually subjected to triglyceride measurements by analytical chemistry (plate assays with the Infinity triglyceride reagent) and pooled together by experimental condition and sent for NMR analysis for VLDL measurement (LipoScience).

Primary hepatocyte apoB studies. Mice of the *Apobec*–/–; *APOB* Tg; *Ldlr*+/– or *Apobec*–/–; *Ldlr*–/– background that had been administered AAV vectors or siRNAs were used as the source of primary hepatocytes for all experiments. Mice were anesthetized with 2,2,2-tribromoethanol and then dissected to expose the liver, portal vein, and inferior vena cava. A catheter was inserted into the portal vein and sutured in place. The livers were perfused with buffer for five minutes to remove all red blood cells, followed by digestion *in situ* by running digestion media through the catheter for 15 minutes. The livers were transferred to 10 mm dishes with 15 ml of hepatocyte wash media and run through a mesh into 50 mL conical tubes to separate the cells. The cells were centifuged at 50 g at 4°C to remove Kupffer cells. The hepatocyte pellets were washed twice with hepatocyte wash media and resuspended in 25 ml PBS + 25 ml of Percoll solution (45 ml Percoll + 5 mL 10x PBS + 100 μl of 1M HEPES). The cells were then

centrifuged at 115 g for five minutes at 4°C to pellet the viable hepatocytes. The hepatocytes were resuspended in Hepatozyme media + 10% FBS + 1% amino acids and plated at one million cells per well. A subset of the cells was analyzed for sortilin and actin protein expression as described above.

For labelling experiments, cells were switched to cystine/methionine-free DMEM with 1% FBS, 1% antibiotics/antimycotics, and 0.4 mM oleic acid for one hour, followed by addition of 200 μCi/well of ^{35}S-methionine/cysteine. After three hours, media from the cells were harvested, and apoB was immunoprecipitated with the antibody ab20737 (Abcam). The immunoprecipitate was subjected to SDS-PAGE, and the gel was exposed to film at –80°C for three days to two weeks. Relative secreted apoB-100 levels were determined by quantitation of appropriately sized bands by densitometry.

To determine relative total secreted protein levels, 50 μl of 2 mg/mL BSA and 25 μl of 50% trichloroacetic acid (TCA) were added to 50 μl of harvested media, followed by incubation on ice for 20 minutes. The samples were centrifuged for 15 minutes, and the pellets were washed with 1 ml of 50% TCA and resuspended by boiling in 1 ml of 0.2 M NaOH. 200 μl of the NaOH suspension was analyzed in a scintillation counter for ^{35}S counts.

Methods References

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SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

Supplementary Figure 1. a, **b**, Mean plasma lipid and lipoprotein particle levels in individuals with zero, one, or two minor alleles of rs646776 in (**a**) MDC-CC and (**b**) PARC. HDL-S = small HDL; HDL-L = large HDL; LDL-VS = very small LDL; LDL-S = small LDL; LDL-M = medium LDL; LDL-L = large LDL; IDL-S = small IDL; IDL-L = large IDL; VLDL-S = small VLDL; VLDL-M = medium VLDL; VLDL-L = large VLDL. All values are in units of nmol/L except LDL-C, which is in mmol/L. Ratios of major allele homozygotes to minor allele homozygotes are normalized to the mean level in minor allele homozygotes. *P* values were derived from logistic regression analyses adjusted for sex, age, and diabetes status. **c**, Sortilin and α-tubulin expression by immunoblot in human liver lysates of various genotypes at rs12740374.

Supplementary Figure 2. a, Associations of 1p13 SNPs genotyped in ~20,000 individuals of European descent and ~9,000 African American individuals with LDL-C. The six SNPs with strongest association in European descendants and the SNP with the strongest association in African Americans are indicated in red. Minor allele frequencies of the SNPs in Europeans (HapMap CEU, release 21), Africans (HapMap YRI, release 21), and African Americans (HapMap ASW, release 27) are shown when available. **b**, Sixteen polymorphisms identified in sequencing of 6.1 kb noncoding DNA region on human BACs harboring the major (Hap1) and minor (Hap2) haplotypes; indicated is whether the polymorphisms are found in HapMap, found in the dbSNP database but not in HapMap, or found in neither.

Supplementary Figure 3. Linkage disequilibrium patterns for SNPs in the vicinity of rs12740374 in Europeans (HapMap CEU, release 21) and Africans (HapMap CEU, release 21). Diagrams were generated with Haploview version 4.1. In each square, the

intensity of red shading is proportional to D′ between SNPs; the number is r^2 between SNPs (if no number is listed, r^2 = 1.0). The six SNPs with strongest association with LDL-C in Europeans are indicated with boxes. Haplotypes for the six SNPs in Europeans and Africans were also generated with Haploview.

Supplementary Figure 4. Firefly luciferase expression from constructs transfected into Hep3B human hepatoma cells. Constructs encode composites and truncations of the 6.1 kb noncoding region shown in Fig. 2a and Supplementary Fig. 5a. Shown are ratios of firefly luciferase expression to Renilla luciferase expression (expressed from cotransfected plasmid), measured 48 hours after transfection, normalized to the ratio from the longest major haplotype construct within each experiment. Restriction enzyme sites for subcloning (BamHI) and PCR-engineered truncations are indicated. Error bars show s.e.m., N = 2 or 3 for each experiment.

Supplementary Figure 5. a, Map of 1p13 SNPs genotyped in ~20,000 individuals of European descent relative to *CELSR2* and *PSRC1* genes. The six SNPs with strongest association with LDL-C (indicated with boxes), comprising a single haplotype, define the 6.1 kb region between the stop codons of the two genes. **b**, Firefly luciferase expression from constructs with haplotypes of 2.1 kb region transfected into Hep3B human hepatoma cells with or without concomitant transduction with A-C/EBP (dominant negative C/EBP) cDNA via lentivirus. Shown are ratios of firefly luciferase expression to Renilla luciferase expression (expressed from cotransfected plasmid), measured 48 hours after transfection, normalized to the ratio from the 2.1 kb major haplotype construct in the absence of A-C/EBP. **c**, Chromatin immunoprecipitation with antibody against C/EBPα in HUES-1 human embryonic stem cells [homozygous minor (TT) at rs12740374] with transduction with C/EBPα cDNA via lentivirus. Immunoprecipitation of DNA sequence surrounding rs12740374 was measured by quantitative PCR, relative to 1:30 dilution of input chromatin, normalized to background (control condition with IgG

beads alone, with no antibody). **d**, Relative *SORT1* expression, determined as a ratio with *B2M* expression by qRT-PCR, in HUES-1 or HUES-9 [homozygous major (GG) at rs12740374] cells either maintained in a pluripotent state or differentiated into endodermal cells with EndoMedia, with or without concomitant transduction C/EBPα cDNA via lentivirus. Error bars show s.e.m., N = 3 for each experiment.

Supplementary Figure 6. Table summarizing the results of all *Sort1* overexpression and knockdown experiments (displayed in Fig. 4, Supplementary Fig. 7, and Supplementary Fig. 9). Each Δ measurement indicates the difference between the experimental mice (mean) and control mice (mean) at the listed time point after injection.

Supplementary Figure 7. a, Sortilin and actin expression by immunoblot in liver and adipose samples from mice receiving AAV8 vectors either containing no gene or murine *Sort1* cDNA. **b**, Plasma samples from *Apobec*–/–; *APOB* Tg mice injected with AAV8 vectors either containing no gene or murine *Sort1* cDNA were subjected: individually to analytical chemistry (Mira) to measure ALT and apoB at baseline, two weeks, and/or six weeks; and as pooled samples to FPLC at two weeks, with full triglyceride profile shown. **c-e**, Plasma samples from (**c**) *Apobec*–/–; *Ldlr*–/– mice, (**d**) *Apobec*–/–; *APOB* Tg; *Ldlr*+/– mice, or (**e**) *Apobec*–/–; *APOB* Tg; *Ldlr*–/– mice injected with AAV8 vectors either containing no gene or murine *Sort1* cDNA were subjected: individually to analytical chemistry (Mira) to measure total cholesterol at baseline and two weeks; and as pooled samples to FPLC to measure LDL-C at two weeks. Error bars show s.e.m.

Supplementary Figure 8. a, Twenty-nine siRNA duplexes designed to target both human and mouse *SORT1* homologues were screened for knockdown of *Sort1* expression in BNL CL.2 cultured mouse embryonic liver cells as assessed by qRT-PCR.

18S rRNA was used as the internal control. Two duplexes displayed greater than 90% knockdown activity. **b**, IC_{50} curve for the duplex with greatest knockdown activity (#22).

Supplementary Figure 9. siRNA duplexes targeting either mouse *Sort1* or the luciferase gene were prepared in lipidoid formulation and administered weekly at 2.0 mg/kg to mice via tail vein injection. Phosphate-buffered saline (PBS) was also used as a control. Plasma samples were collected before the first injection and, depending on the experiments, at three days, five days, and/or two weeks after the first injection. **a**, *Sort1* mRNA levels were measured from liver tissue with branched DNA assay. **b**, Rapid amplification of cDNA ends (5′-RACE) in liver samples from mice injected with siRNA duplexes at the indicated doses confirming siRNA-mediated cleavage specifically with *Sort1* duplex. An siRNA duplex results in cleavage of its target mRNA sequence 10 bp from the 5′-end of the antisense strand; attachment of an adaptor oligonucleotide to RNAs isolated from liver, reverse transcription (RT) with a *Sort1*-specific primer, and PCR with the one primer positioned between the RT primer and the expected cleavage site and the other primer matching the adaptor should yield product of a fixed size (corresponding to the distance between the first PCR primer and the cleavage site) only if there is on-target siRNA-mediated cleavage. PCR products of the predicted size were obtained only from mice receiving *Sort1* duplex. 92% of subcloned products had the expected *Sort1* sequences (data not shown). Of note, this study used a different lipidoid formulation than the other experiments, and so the siRNA doses are not equivalent. **c**, Sortilin and actin expression by immunoblot in liver and adipose samples from mice receiving control or *Sort1* siRNA injections. **d**, Plasma samples from *Apobec*–/–; *APOB* Tg mice injected with PBS or *Sort1* siRNA were subjected: individually to analytical chemistry (Mira) to measure apoB at baseline and two weeks; and as pooled samples to FPLC at two weeks, with the full triglyceride profile shown. **e-g**, Plasma samples from (**e**) *Apobec*–/–; *Ldlr*–/– mice or (**f**, **g**) *Apobec*–/–; *APOB* Tg; *Ldlr*+/– mice injected with

PBS or luciferase siRNA or *Sort1* siRNA were subjected: individually to analytical chemistry (Mira) to measure total cholesterol at baseline, three days, five days, or two weeks; as pooled samples to FPLC to measure LDL-C at three days or two weeks; and individually to NMR to measure VLDL particle concentrations at five days. **h**, Plasma samples from *Sort1*–/– mice or wild-type mice were subjected: individually to analytical chemistry (Mira) to measure total cholesterol and as pooled samples to FPLC to measure LDL-C. Error bars show s.e.m.

Supplementary Figure 10. Primary mouse hepatocytes from *Apobec1*–/–; *APOB* Tg; *Ldlr*+/– mice (**a**-**d**, **f**, **g**) or *Apobec1*–/–; *Ldlr*–/– mice (**e**) were labeled for three hours, followed by collection of media, immunoprecipitation of apoB, polyacrylamide gel electrophoresis, and quantitation of radioactive counts from bands corresponding to apoB-100, as well as counts from trichloroacetic acid precipitation of media to determine total secreted protein levels. ApoB-100 measurements were standardized to total secreted protein measurements. **a**, Labeled apoB-100 secretion from hepatocytes receiving siRNA duplexes targeting luciferase or mouse *Sort1*. **b**, Sortilin and actin expression by immunoblot for experiment shown in **a**. **c**, Autoradiograph for experiment shown in **a**. **d**, **e**, Labeled apoB-100 secretion from hepatocytes infected with adeno-associated virus 8 (AAV8) vectors either containing no gene or murine *Sort1* cDNA. **f**, Sortilin and actin expression by immunoblot for experiment shown in **d**. The blot was intentionally underexposed to best represent the difference in sortilin expression. **g**, Autoradiograph for experiment shown in **d**.

Supplementary Figure 1.

a

Malmö Diet and Cancer Study – Cardiovascular Cohort (ion mobility)

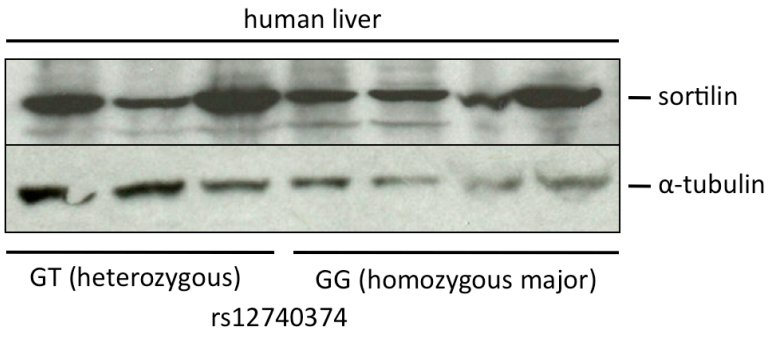
Minor alleles	N	HDL-S	HDL-L	LDL-VS	LDL-S	LDL-M	LDL-L	IDL-S	IDL-L	VLDL-S	VLDL-M	VLDL-L	LDL-C
0	2689	2972	1633	114	82.3	126	441	121	216	54.3	36.7	9.47	4.23
1	1607	2913	1624	104	79.1	121	424	116	213	52.3	35.6	9.18	4.08
2	279	3067	1690	94.8	74.7	116	416	115	213	53.2	35.7	9.05	3.97
Ratio		0.97	0.97	1.20	1.10	1.09	1.06	1.05	1.01	1.02	1.03	1.05	1.07
P value		0.84	0.75	1.1×10^{-11}	0.03	0.02	0.0004	0.0002	0.24	0.006	0.02	0.04	2.4×10^{-11}

b

Pharmacogenomics and Risk of Cardiovascular Disease study (gradient gel electrophoresis)

Minor alleles	N	LDL-VS	LDL-S	LDL-M	LDL-L	LDL-C
0	1196	14.4	17.1	26.4	56.3	3.45
1	589	12.5	16.7	26.1	54.5	3.35
2	75	10.5	14.2	24.5	58.4	3.26
Ratio		1.37	1.20	1.08	0.96	1.06
P value		8.0×10^{-11}	0.16	0.48	0.29	0.004

c



Supplementary Figure 2.

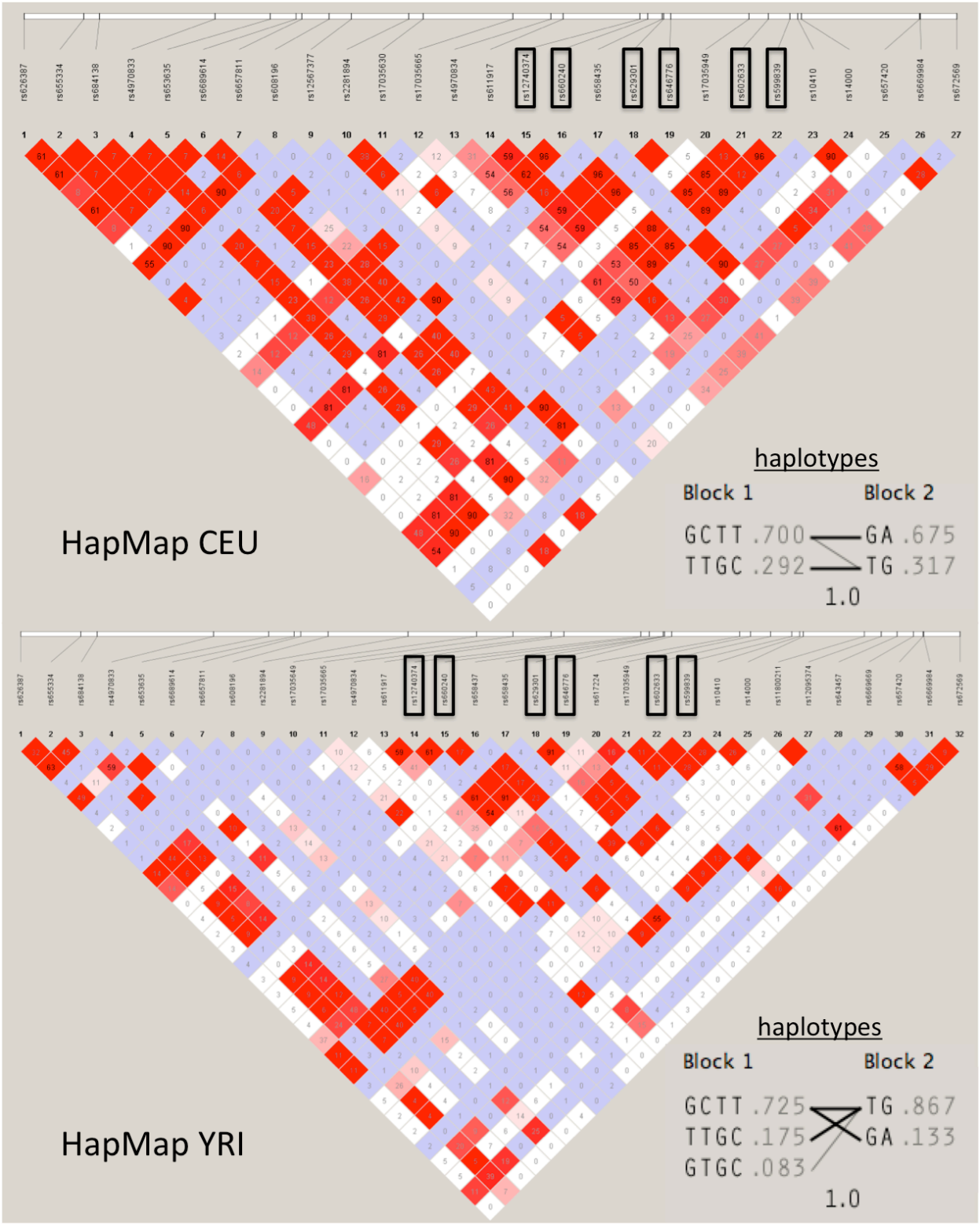
a

SNP	*P* value Europeans	*P* value African Americans	MAF CEU	MAF YRI	MAF ASW
rs4970833	4.2×10^{-11}	0.12	0.417	0.058	0.119
rs653635	0.12		0.092	0.275	0.190
rs6689614	4.6×10^{-11}	0.12	0.417	0.058	0.119
rs6657811	3.3×10^{-22}	3.3×10^{-7}	0.167	0.133	—
rs608196	0.098		0.083	0.008	0.032
rs2281894	7.2×10^{-5}	0.08	0.225	0.017	—
rs17035630	0.0066	0.94	0.100	0.100	0.040
rs17035665	0.00047		0.175	0.217	—
rs4970834	1.6×10^{-25}	0.002	0.275	0.325	0.254
rs611917	8.9×10^{-29}	9.2×10^{-15}	0.375	0.275	0.413
rs12740374	**1.8×10^{-42}**	**2.3×10^{-20}**	0.300	0.183	—
rs660240	**8.3×10^{-41}**		0.292	0.267	0.429
rs658435	0.023	0.18	0.092	0.100	0.079
rs629301	**2.2×10^{-41}**		0.300	0.267	0.429
rs646776	**2.2×10^{-41}**	1.6×10^{-13}	0.300	0.267	0.437
rs17035949	1.0×10^{-6}	0.02	0.058	0.425	0.302
rs602633	**7.6×10^{-41}**	0.05	0.317	0.133	—
rs599839	**7.3×10^{-42}**	0.03	0.325	0.133	0.238
rs10410	0.057	0.62	0.092	0.042	—
rs14000	0.062	0.86	0.100	0.142	0.119
rs657420	1.3×10^{-9}	0.003	0.483	0.183	0.238
rs672569	2.0×10^{-14}		0.233	0.433	0.468

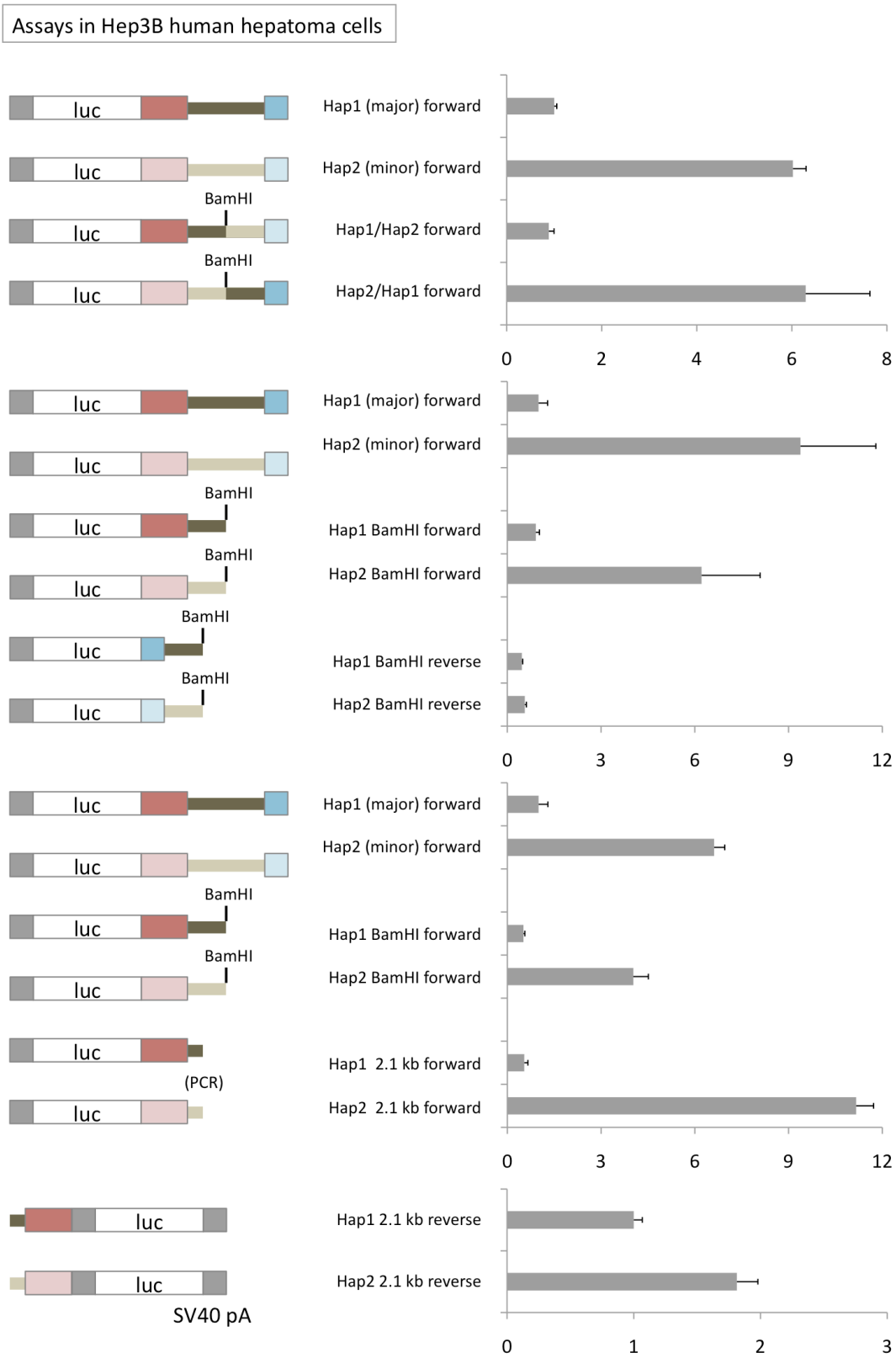
b

Variant	Hap1 allele	Hap2 allele
Novel 1-base indel	—	C
rs7528419 (HapMap)	A	G
rs11102967 (dbSNP)	T	C
rs12740374 (HapMap)	G	T
rs660240 (HapMap)	C	T
rs3832016 (dbSNP)	T	—
rs629301 (HapMap)	T	G
rs646776 (HapMap)	T	C
Novel SNP	T	C
rs3902354 (dbSNP)	A	C
Novel SNP	A	G
rs583104 (dbSNP)	T	G
rs602633 (HapMap)	G	T
rs4970837 (dbSNP)	T	G
rs1277930 (dbSNP)	A	G
rs599839 (HapMap)	A	G

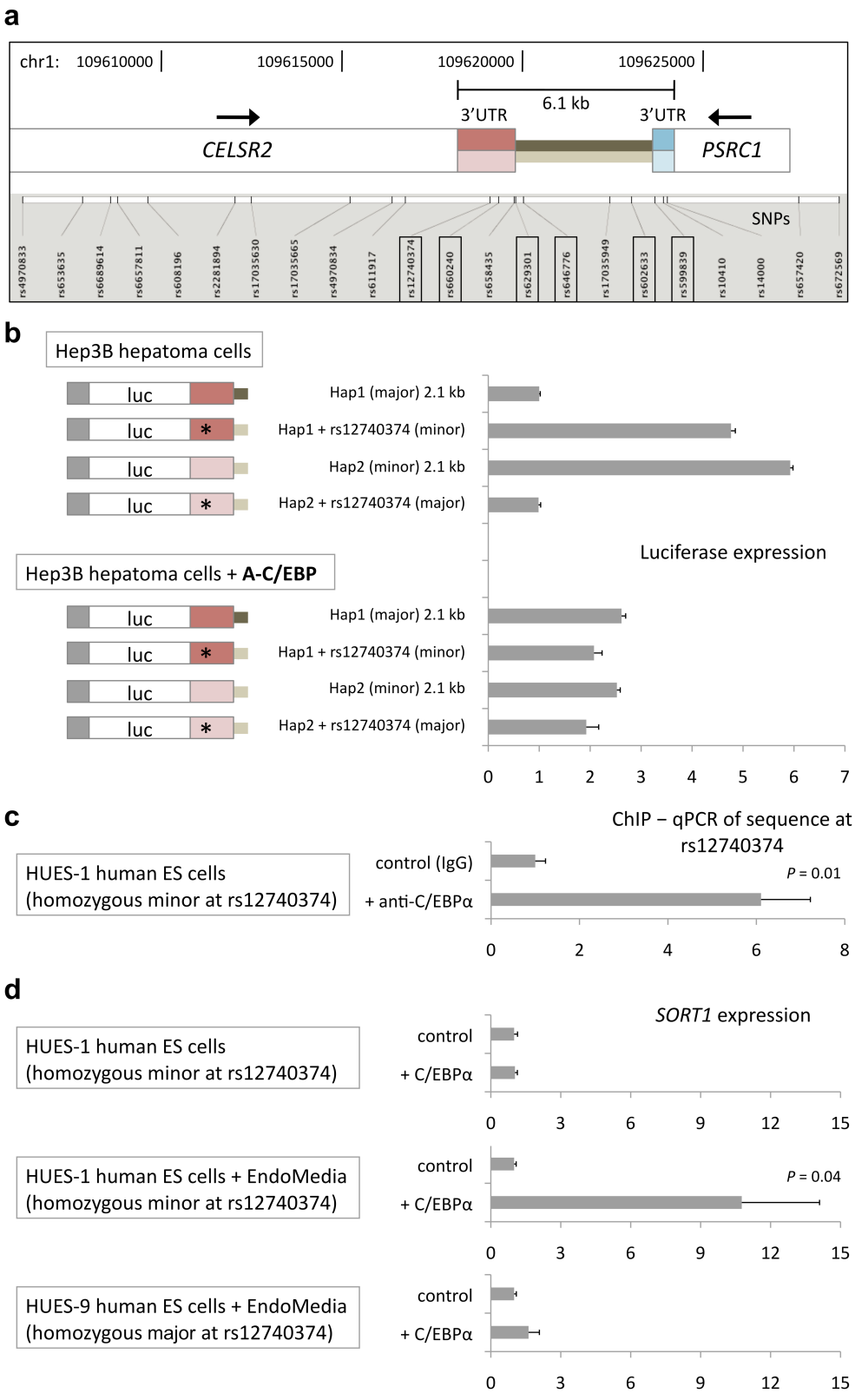
Supplementary Figure 3.



Supplementary Figure 4.



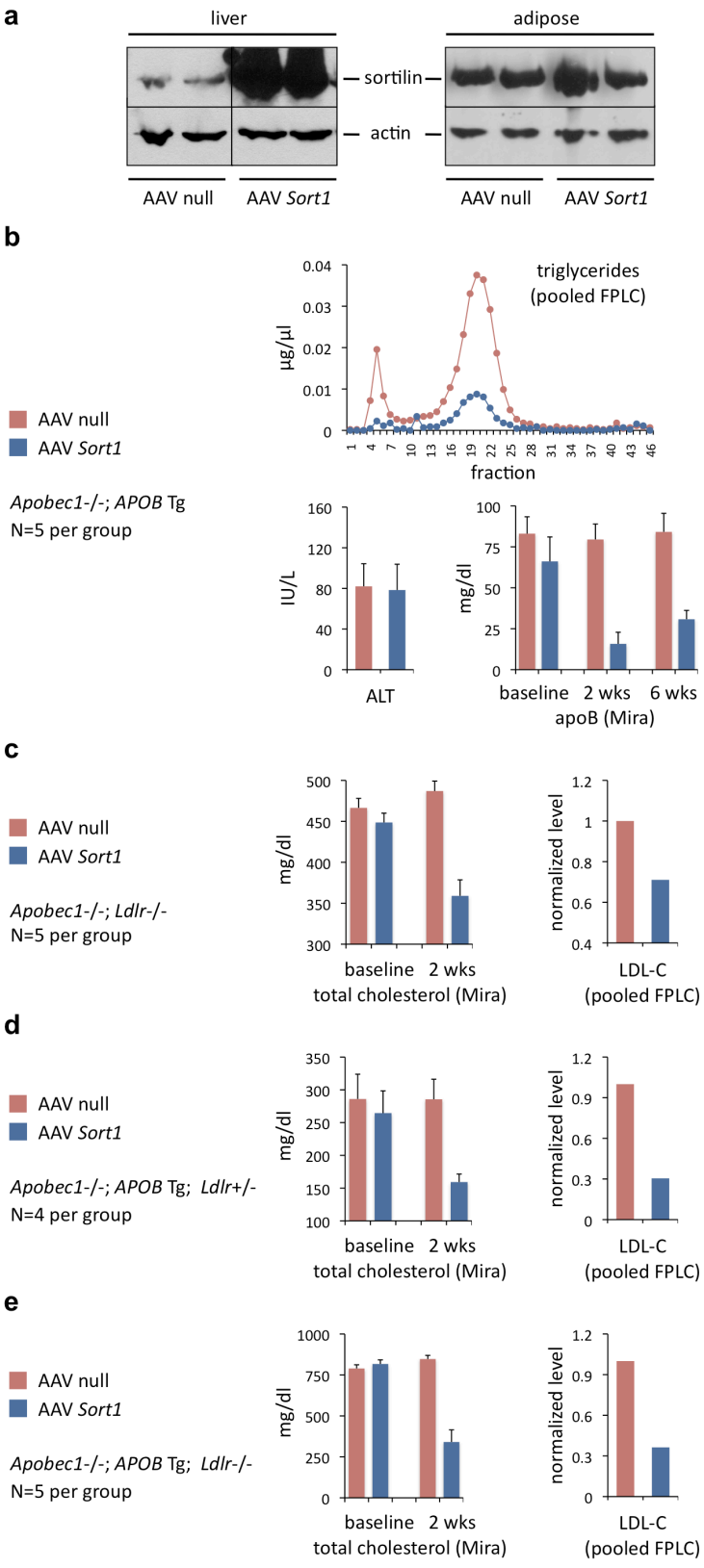
Supplementary Figure 5.



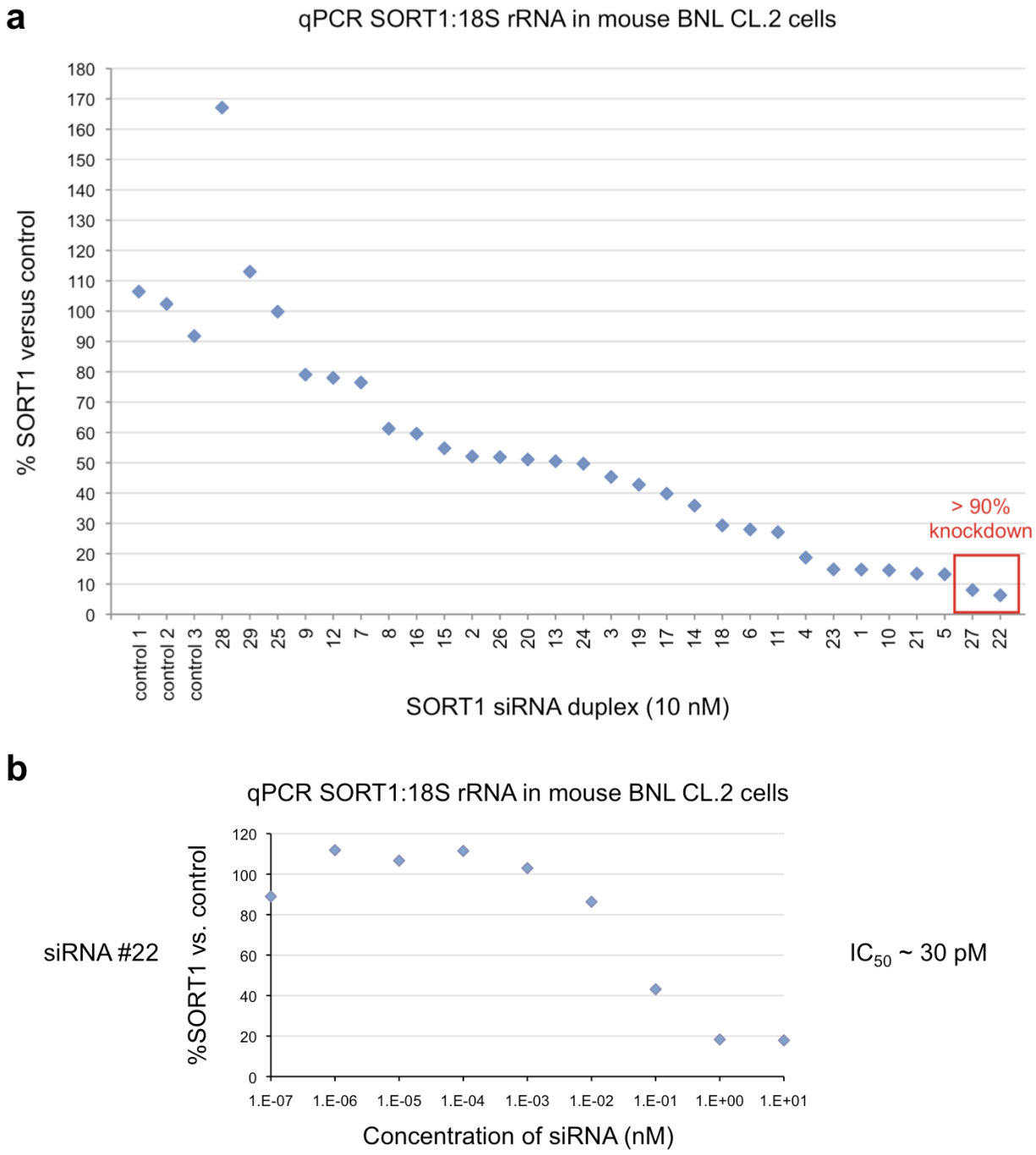
Supplementary Figure 6.

genetic background	control	experimental	Δ total chol. (Mira)	Δ LDL-C (FPLC)
		***Sort1* overexpression**		
Apobec1-/-; *APOB* Tg	AAV null	AAV *Sort1*	–70% (2 weeks) –46% (6 weeks)	–73% (2 weeks)
Apobec1-/-; *Ldlr-/-*	AAV null	AAV *Sort1*	–26% (2 weeks)	–29% (2 weeks)
Apobec1-/-; *APOB* Tg; *Ldlr+/-*	AAV null	AAV *Sort1*	–44% (2 weeks)	–70% (2 weeks)
Apobec1-/-; *APOB* Tg; *Ldlr-/-*	AAV null	AAV *Sort1*	–60% (2 weeks)	–64% (2 weeks)
		***Sort1* knockdown**		
Apobec1-/-; *APOB* Tg	PBS	*Sort1* siRNA	+46% (2 weeks)	+125% (2 weeks)
Apobec1-/-; *Ldlr-/-*	PBS	*Sort1* siRNA	+21% (3 days) +20% (5 days)	+16% (5 days)
	luciferase siRNA		+22% (3 days) +24% (5 days)	+22% (5 days)
Apobec1-/-; *APOB* Tg; *Ldlr+/-*	PBS	*Sort1* siRNA	+25% (2 weeks)	+49% (2 weeks)
Apobec1-/-; *APOB* Tg; *Ldlr+/-*	luciferase siRNA	*Sort1* siRNA	+24% (5 days) +22% (2 weeks)	+32% (5 days) +29% (2 weeks)

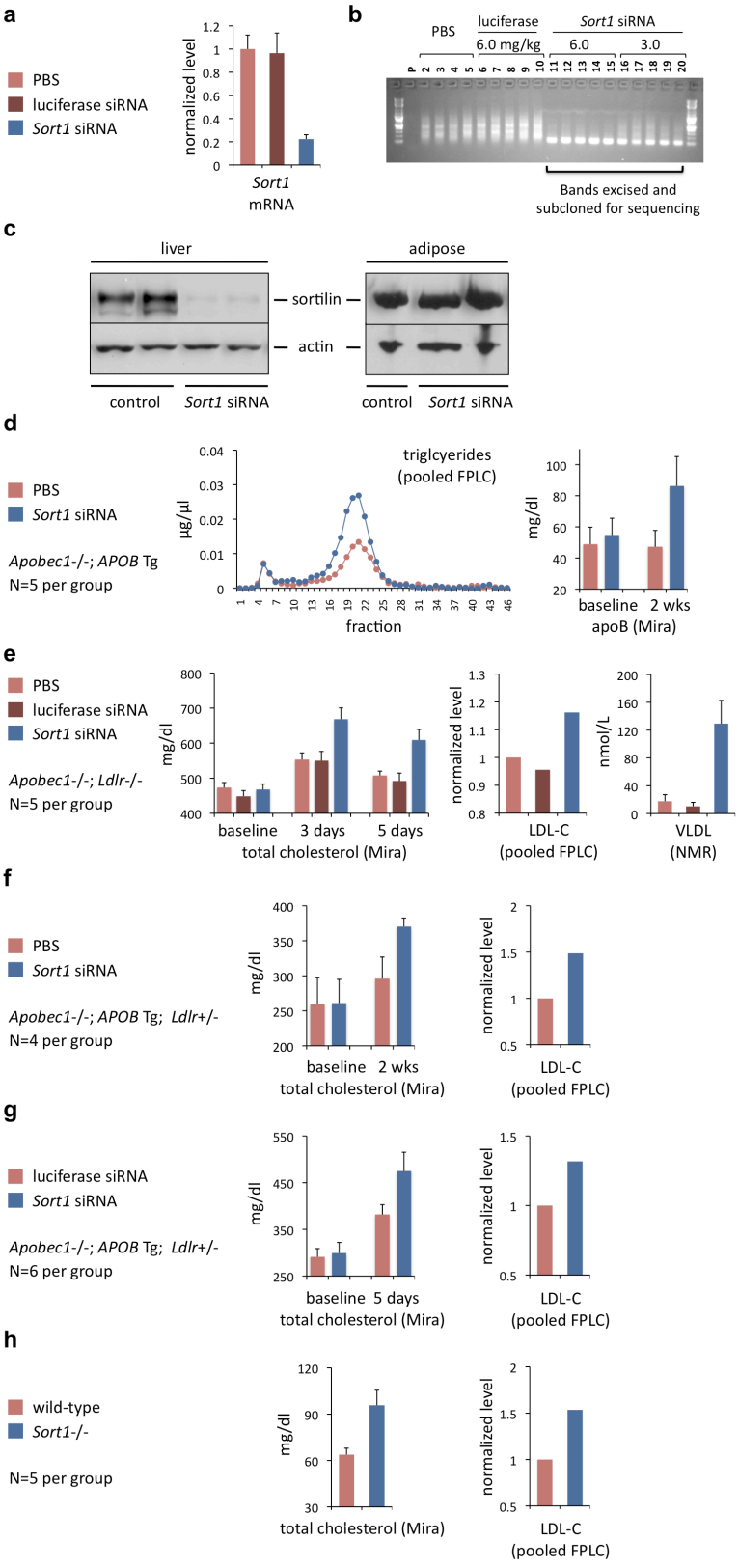
Supplementary Figure 7.



Supplementary Figure 8.



Supplementary Figure 9.



Supplementary Figure 10.