SUPPLEMENTARY INFORMATION

a

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

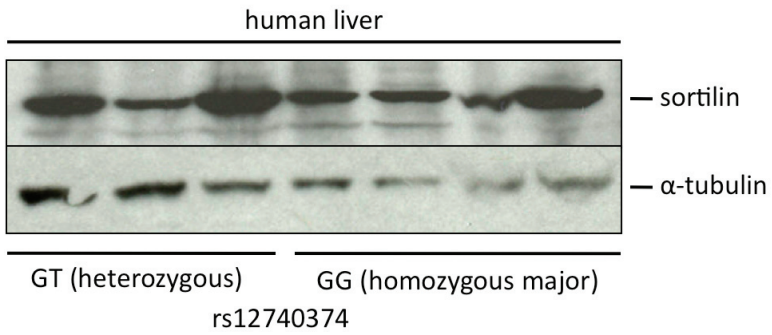
Minor alleles	N	HDL-S	HDL-L	LDL-VS	LDL-S	LDL-M	LDL-L	IDL-S	IDL-L	VLDL-S	VLDL-M	VLDL-L	LDL-C
0	2689	2972	1633	114	82.3	126	441	121	216	54.3	36.7	9.47	4.23
1	1607	2913	1624	104	79.1	121	424	116	213	52.3	35.6	9.18	4.08
2	279	3067	1690	94.8	74.7	116	416	115	213	53.2	35.7	9.05	3.97
Ratio		0.97	0.97	1.20	1.10	1.09	1.06	1.05	1.01	1.02	1.03	1.05	1.07
P value		0.84	0.75	$1.1x10^{-11}$	0.03	0.02	0.0004	0.0002	0.24	0.006	0.02	0.04	$2.4x10^{-11}$

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		$8.0x10^{-11}$	0.16	0.48	0.29	0.004

c



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.

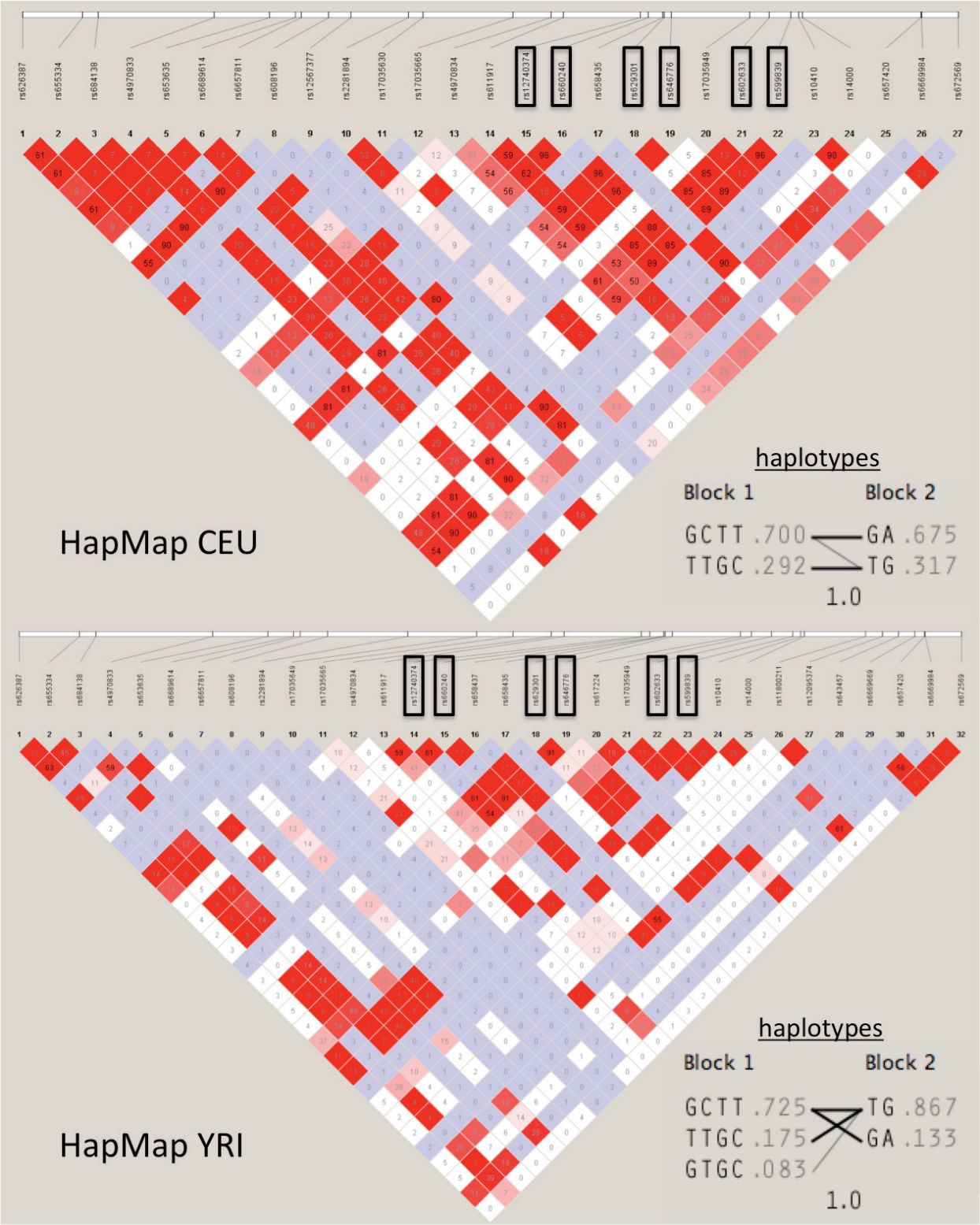
a

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

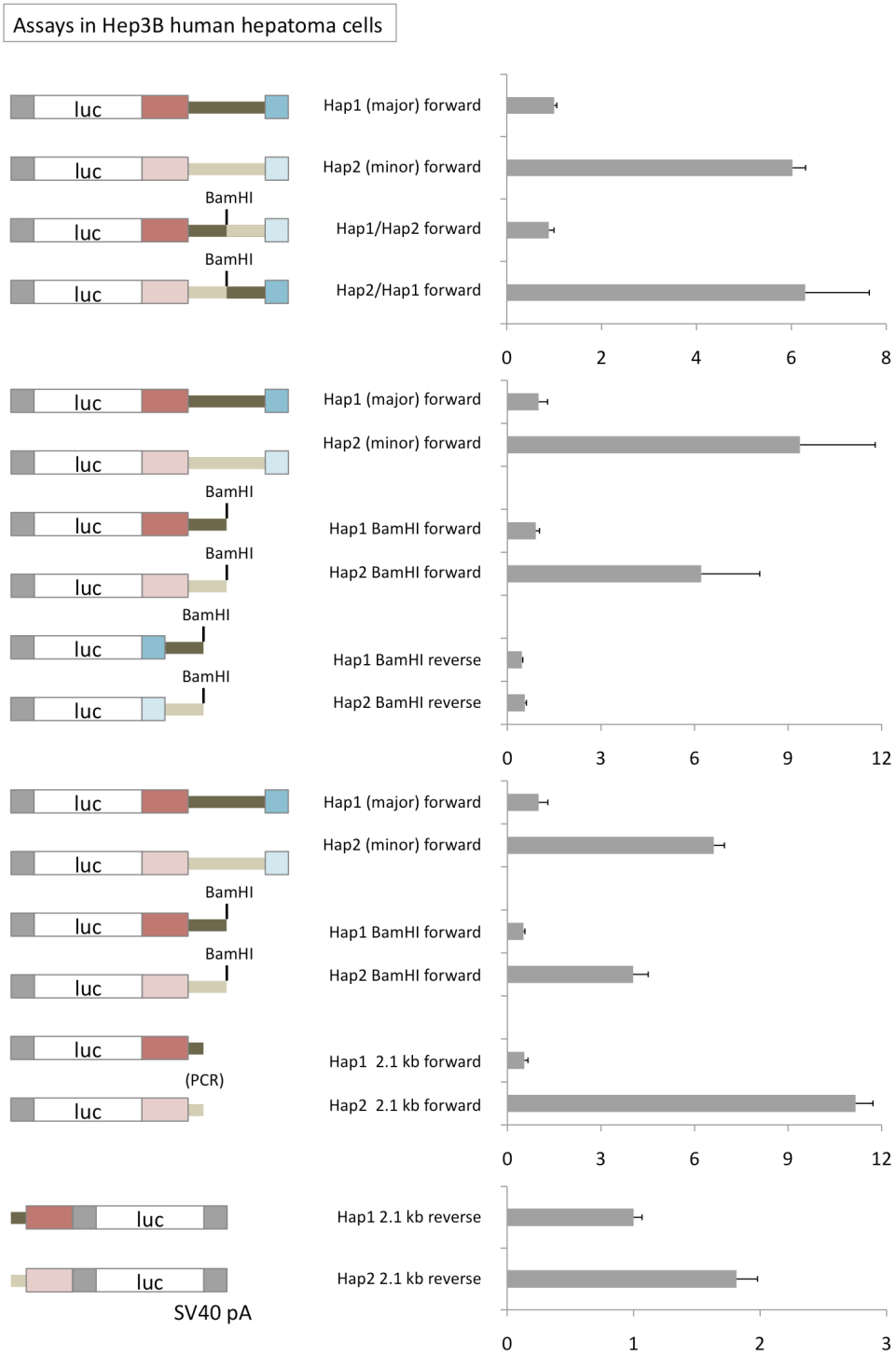
b

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

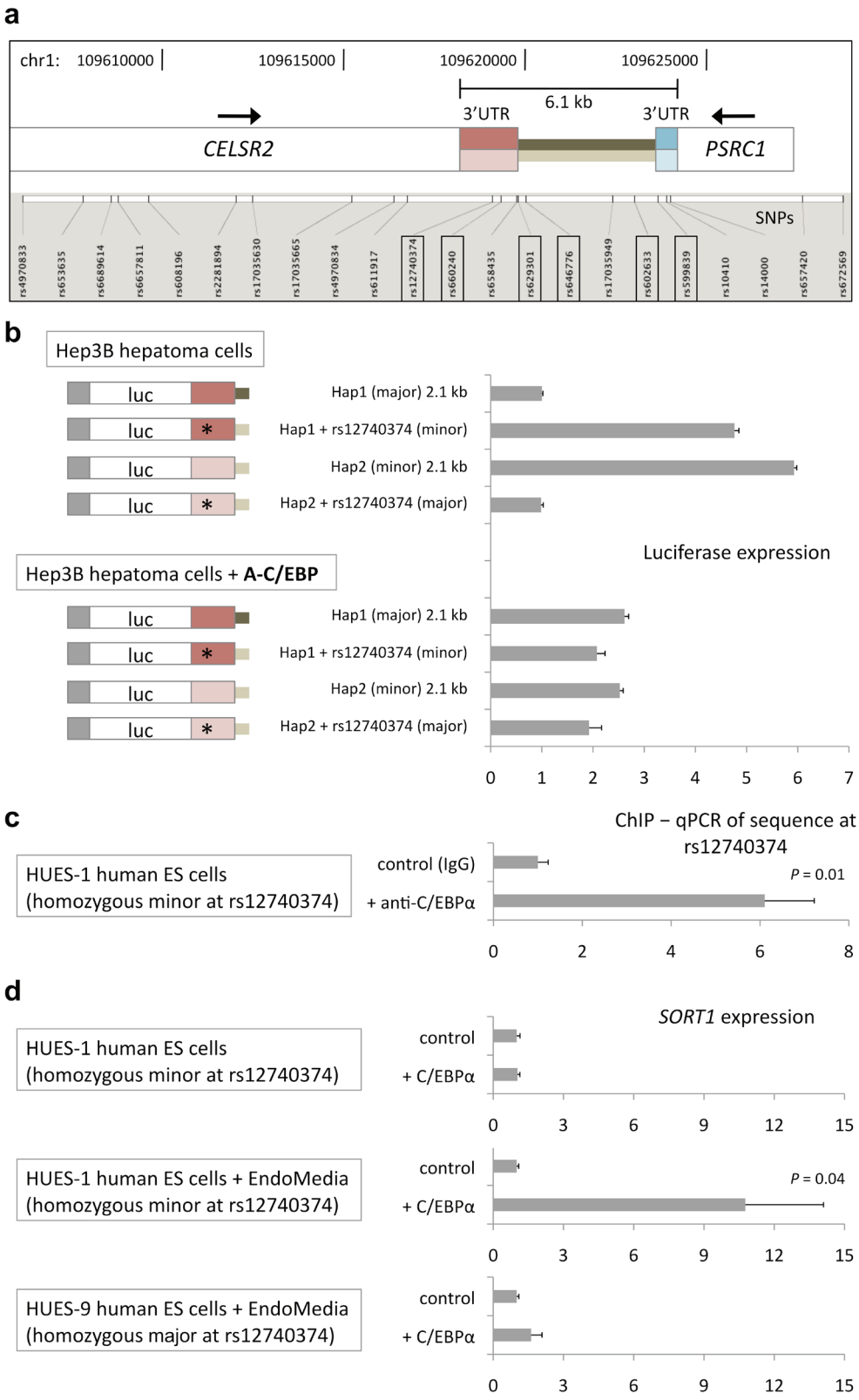
Supplementary Figure 2. a, Associations of 1p13 SNPs genotyped in ~20,000 individuals of European descent and ~9,000 African American individuals with LDL-C. The six SNPs with strongest association in European descendants and the SNP with the strongest association in African Americans are indicated in 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.

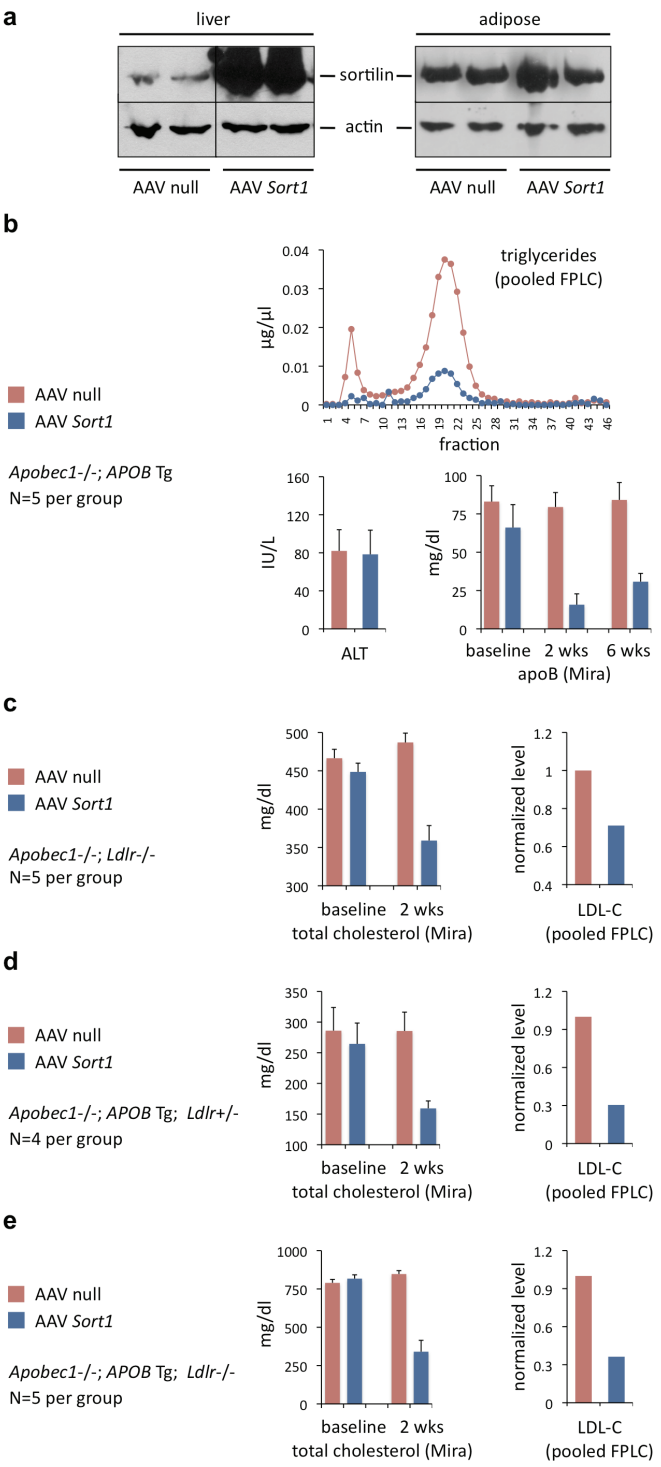


Supplementary Figure 5. a, Map of 1p13 SNPs genotyped in ~20,000 individuals of European descent relative to *CELSR2* and *PSRC1* genes. The six SNPs with strongest association with LDL-C (indicated with boxes), comprising a single haplotype, define the 6.1 kb region between the stop codons of the two genes. **b**, Firefly luciferase expression from constructs with haplotypes of 2.1 kb region transfected into Hep3B human

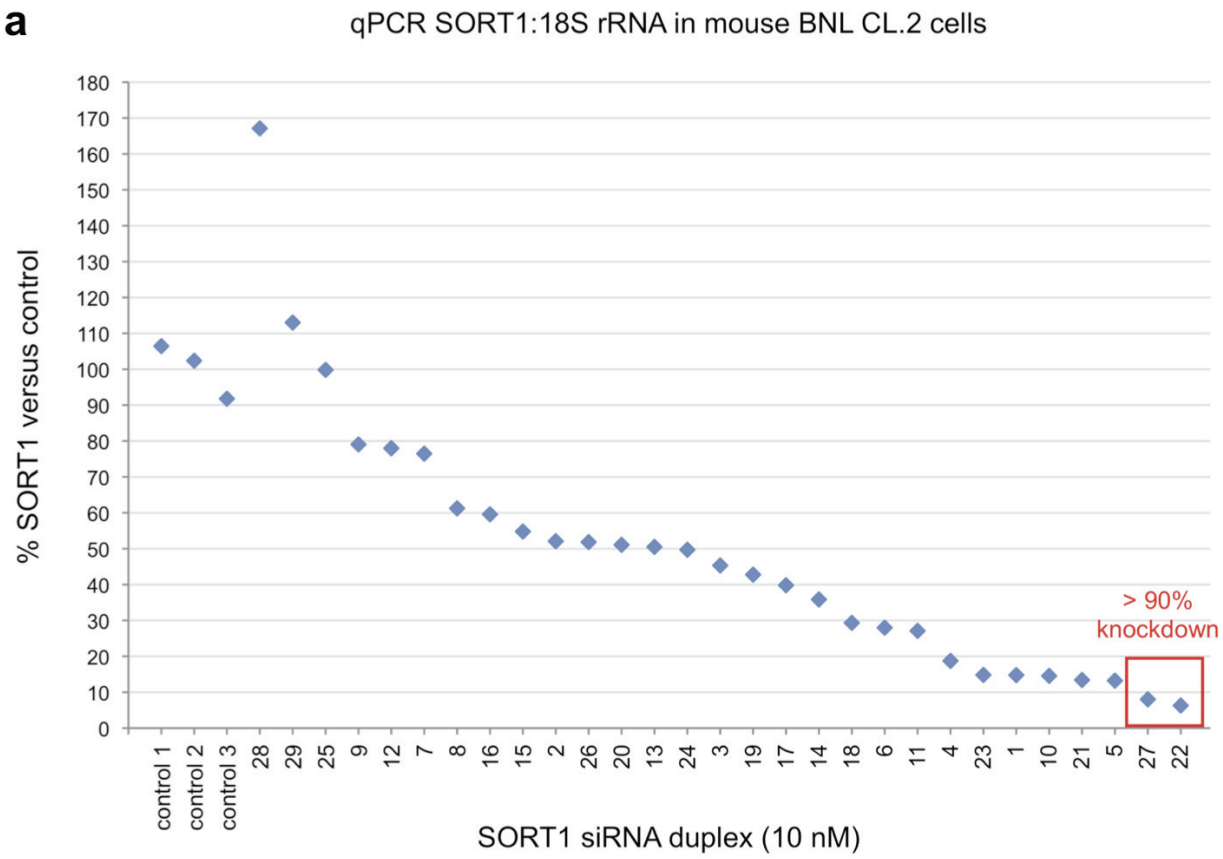
hepatoma cells with or without concomitant transduction with A-C/EBP (dominant negative C/EBP) cDNA via lentivirus. Shown are ratios of firefly luciferase expression to Renilla luciferase expression (expressed from cotransfected plasmid), measured 48 hours after transfection, normalized to the ratio 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.

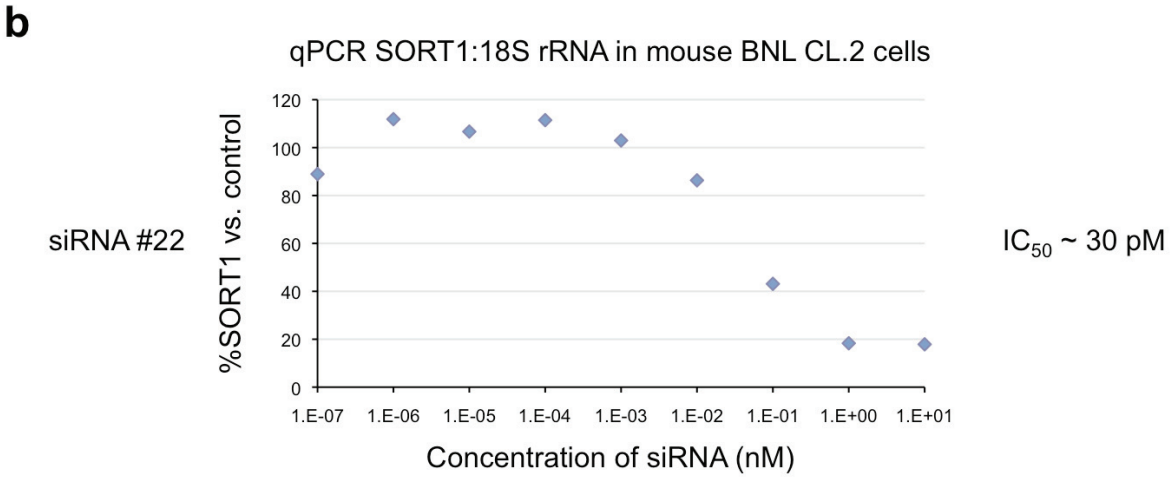
genetic background	control	experimental	Δ total chol. (Mira)	Δ LDL-C (FPLC)
		***Sort1* overexpression**		
Apobec1-/-; APOB Tg	AAV null	AAV *Sort1*	–70% (2 weeks) –46% (6 weeks)	–73% (2 weeks)
Apobec1-/-; Ldlr-/-	AAV null	AAV *Sort1*	–26% (2 weeks)	–29% (2 weeks)
Apobec1-/-; APOB Tg; *Ldlr+/-*	AAV null	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 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.

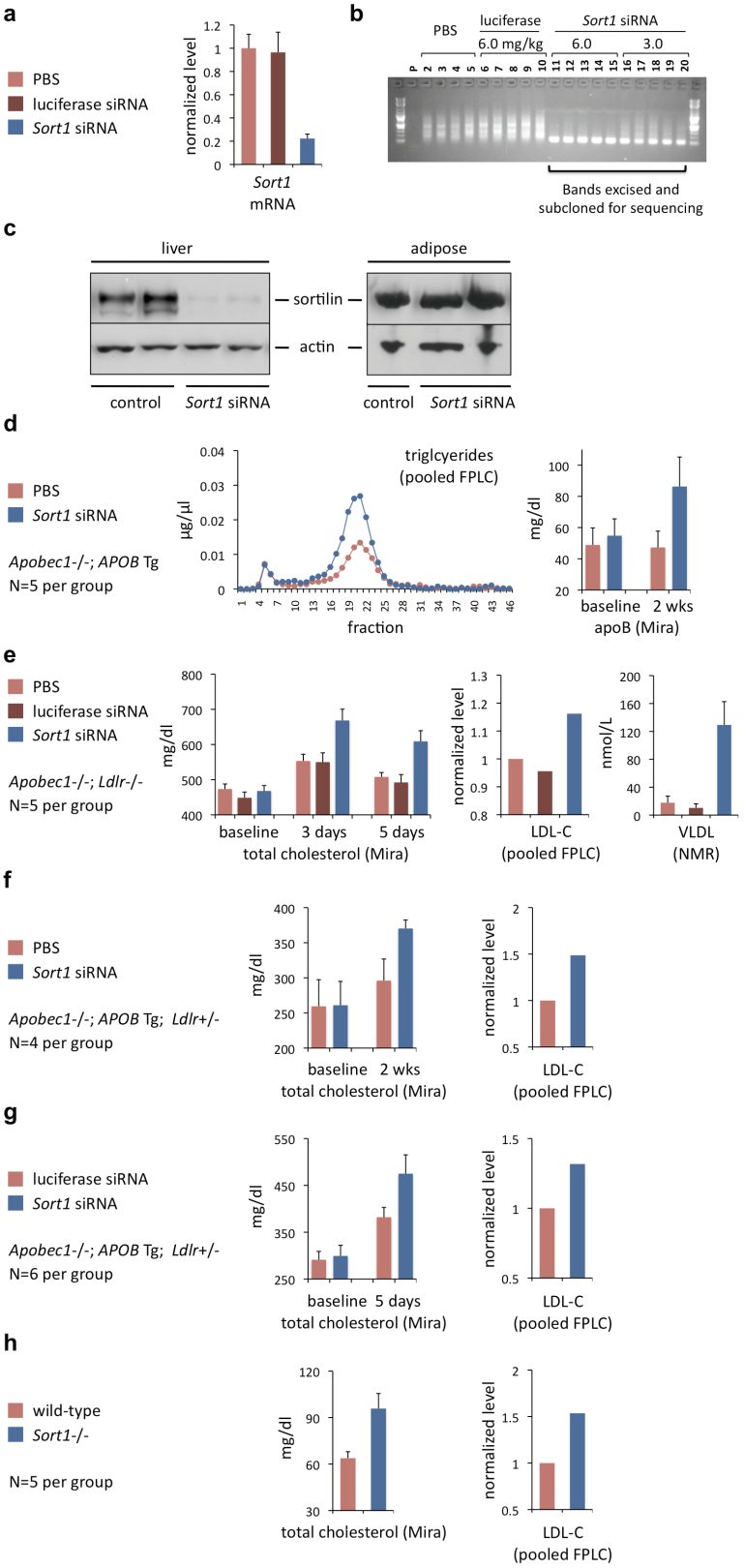


Supplementary Figure 7. a, Sortilin and actin expression by immunoblot in liver and adipose samples from mice receiving AAV8 vectors either containing no gene or murine *Sort1* cDNA. **b**, Plasma samples from *Apobec*–/–; *APOB* Tg mice injected with AAV8 vectors either containing no gene or murine *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.



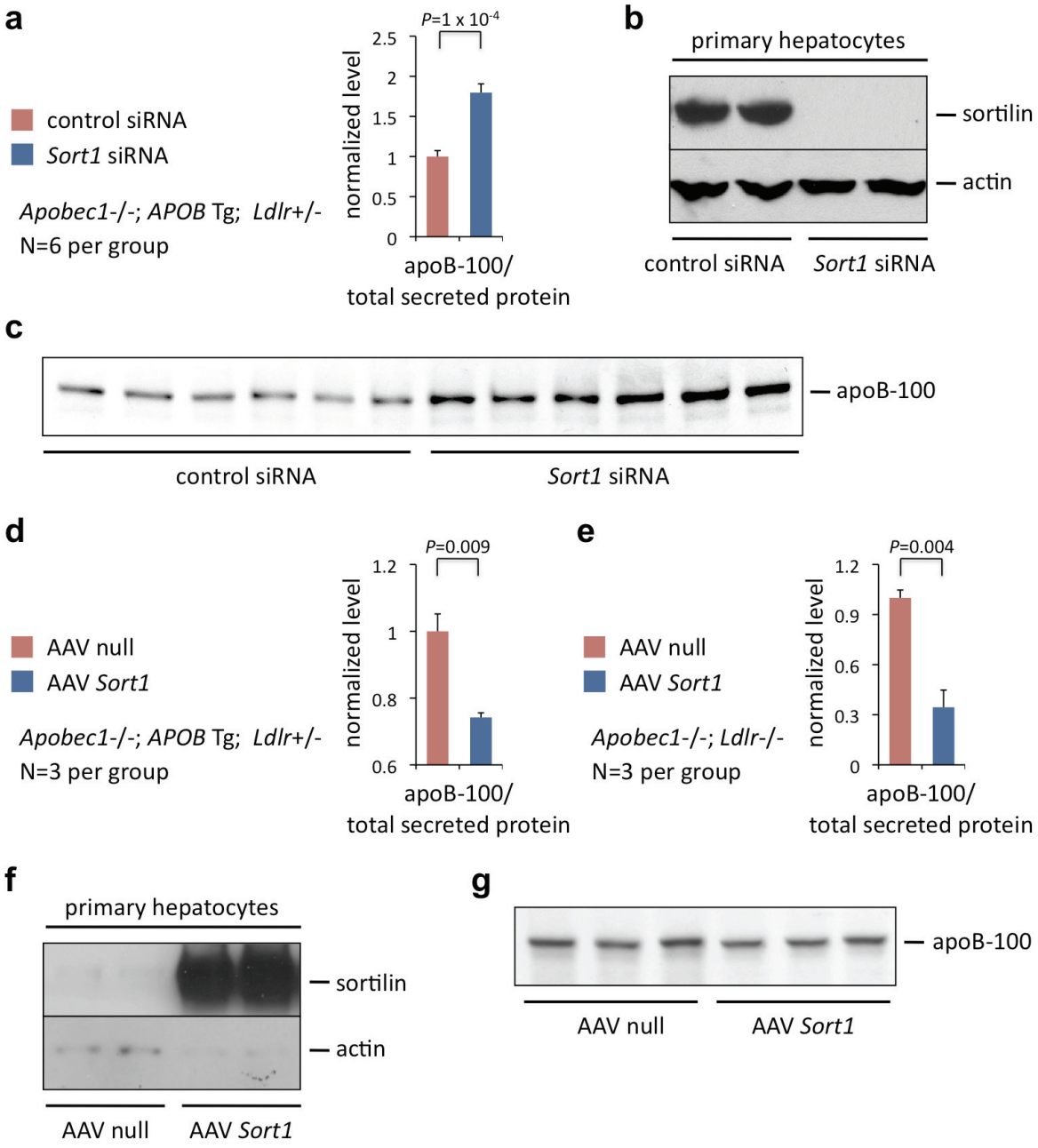


Supplementary Figure 8. a, Twenty-nine siRNA duplexes designed to target both human and mouse *SORT1* homologues were screened for knockdown of *Sort1* expression in BNL CL.2 cultured mouse embryonic liver cells as assessed by qRT-PCR. 18S rRNA was used as the internal control. Two duplexes displayed 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**.