Supplementary Data

Sortilin enhances secretion of apolipoprotein(a) through effects on apolipoprotein B secretion and promotes uptake of lipoprotein(a)

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Table S1. Primers used in constructing sortilin expression plasmids[a]

Trafficking Mutants	**Primer Sequence**
SORT1-Y792A	5'-GTT CCT GGT GCA TCG A**GC** CTC TGT GCT GCA GCA G-3'
SORT1-L829A/L830A	5'-GAT GAC TCA GAT GAG GAC **GC**C **GC**G GAA CCT CGA GGT CAC CC-3'
Polymorphic Variants	
SORT1-I124V	5'-CACTGGGGTC**G**TTCTAGTCTTG-3'
SORT1-K205N	5'-ATTTTGCGAA**T**AATTTTGTGCAAAC-3'
SORT1-K302E	5'-TATTGGTGTG**G**AAATCTACTCATTTG-3'
SORT1-F404Y	5'-GAGACGGACT**A**TACCAACGTG-3'
SORT1-E444Q	5'-GAGGAAGCCT**C**AAAACAGTGAATGTG-3'
SORT1-E447G	5'-GAAAACAGT**GG**ATGTGATGCTACAGC-3'
SORT1-V650M	5'-CAAGTCATCC**AT**GTGTCAGAATGG-3'

[a]Sense strand sequences only are shown. Mutated codons are double underlined; mutated nucleotides are in boldface type. The 5′-end of the antisense primer was designed at the base next to the 5′-end of the sense primer, and preceded in the opposite direction on the complementary strand.

Table S2. *SORT1* rare nonsynonymous missense and splicing variants identified among individuals with Lp(a) >30 mg/dL

cDNA variation: amino acid change or splice site change	Chromosome 1 position	Reference allele	Alternate allele	Variant type
SORT1 exon 1: c.C10T:p.P4S	1:109940505	G	A	nonsynonymous SNV
SORT1 exon 1: c.C145G:p.R49G	1:109940370	G	C	nonsynonymous SNV
SORT1 exon 1: c.G28T:p.G10C	1:109940487	C	A	nonsynonymous SNV
SORT1 exon 3: c.307-11T>-	1:109912222	A	-	splicing
SORT1 exon 3: c.A370G:p.I124V†	1:109910100	T	C	nonsynonymous SNV
SORT1 exon 3: c.G381C:p.L127F	1:109910089	C	G	nonsynonymous SNV
SORT1 exon 5: c.G615T:p.K205N†	1:109897082	C	A	nonsynonymous SNV
SORT1 exon 8: c.A904G:p.K302E†	1:109888432	T	C	nonsynonymous SNV
SORT1 exon 9: c.G1072T:p.D358Y	1:109884672	C	A	nonsynonymous SNV
SORT1 exon 10: c.T1211A:p.F404Y†	1:109883399	A	T	nonsynonymous SNV
SORT1 exon 11: c.A1340G:p.E447G†	1:109878893	T	C	nonsynonymous SNV
SORT1 exon 11: c.G1330C:p.E444Q†	1:109878903	C	G	nonsynonymous SNV
SORT1 exon 14: c.C1682T:p.T561M	1:109867673	G	A	nonsynonymous SNV
SORT1 exon 14: c.1475-4G>T	1:109869786	C	A	splicing
SORT1 exon 15: c.G1948A:p.V650M†	1:109865630	C	T	nonsynonymous SNV
SORT1 exon 17: c.2025-7C>G	1:109860598	G	C	splicing

Reference gene sequence for *SORT1* is NM_002959. All variants have minor allele frequency <0.01.
Chromosome 1 position based on human genome build hg38.
Abbreviations: cDNA, coding DNA; SNV, single nucleotide variant
†identified in subjects with Lp(a) levels in 95th percentile

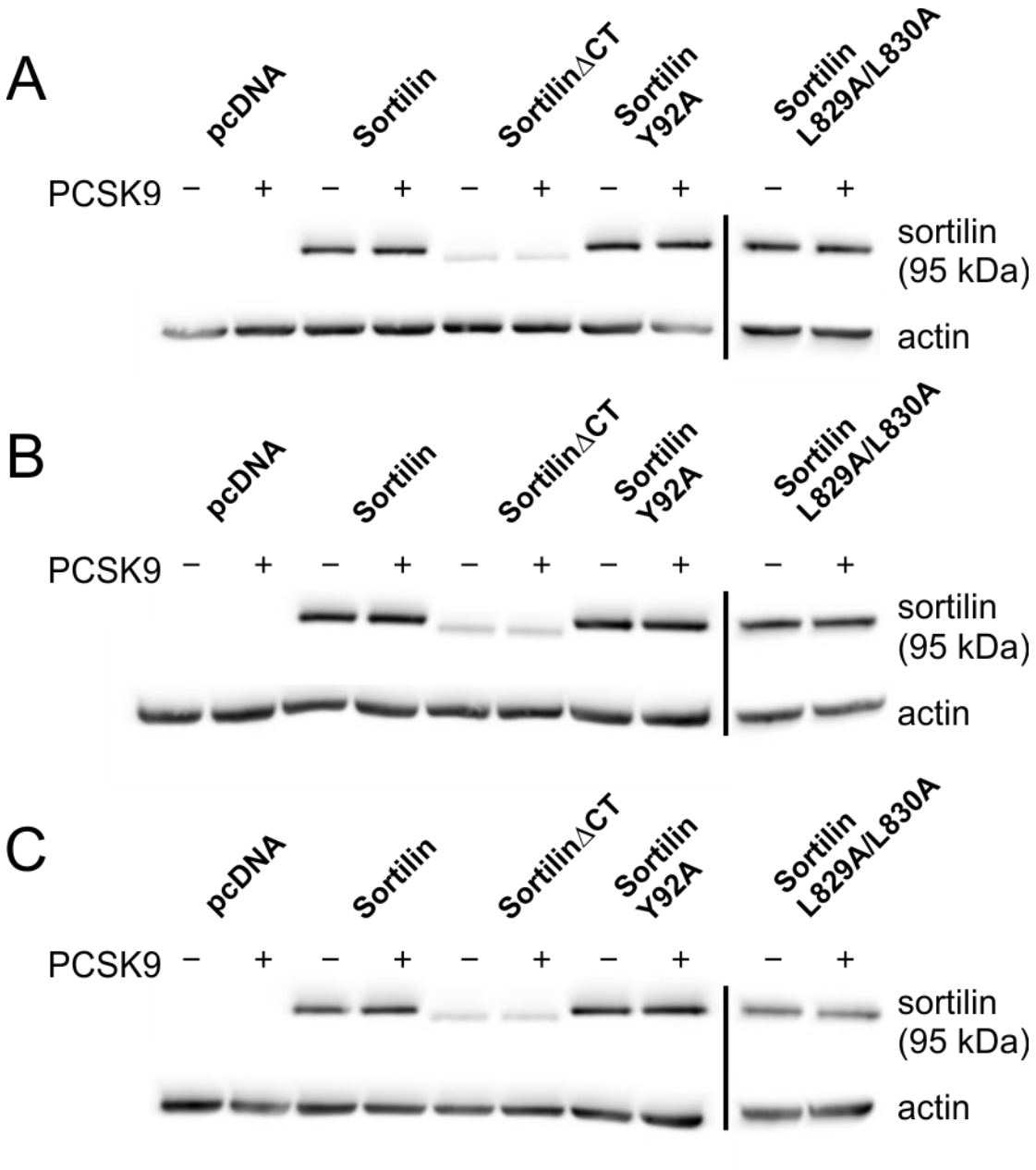

Figure S1. Expression of sortilin trafficking variants. HepG2 cells were transiently transfected with expression plasmids encoding the indicated variants or the empty expression plasmid pcDNA. All variants contained a carboxyl-terminal myc tag. Lysates were prepared after 24 incubation with 17K apo(a) (200 nM) (A) or Lp(a) (10 µg/mL) (B,C) in the presence or absence of 20 µg/mL PCSK9. Lysates were prepared and subjected to western blot analysis with mouse-anti-human c-Myc epitope tag antibodies to visualize ectopically expressed sortilin or mouse anti-human actin antibodies. Note that these expression patterns are also representative of observations in pulse-chase secretion experiments.

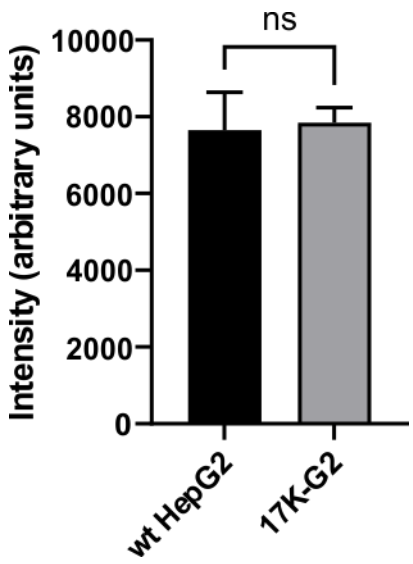

Figure S2. Expression of apoB in HepG2 cells or 17K-HepG2 cells. Cells were incubated for 1 hour in the presence of 0.4 mM oleic acid. Medium samples were subjected to western blot analysis using a goat polyclonal anti-apoB antibody (1:5000 dilution). ApoB expression was quantified by densitometric analysis of the blots. The data shown are the means ± standard deviation of 3 independent medium preparations for each cell line. ns: non-significant ($p>0.05$ by unpaired Student's t-test).

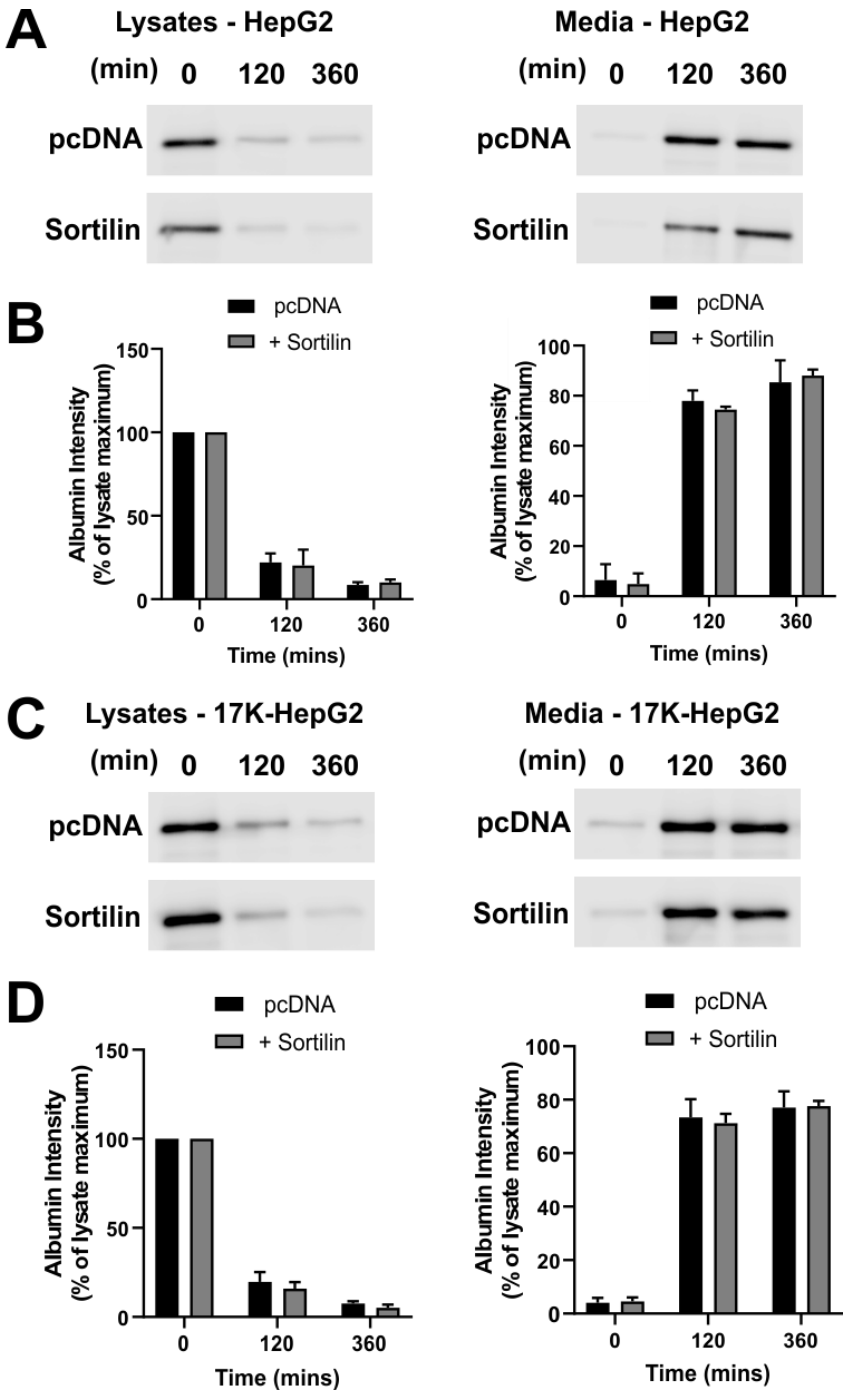

Figure S3. Sortilin overexpression does not affect secretion of albumin from hepatocytes. Wild-type HepG2 cells (A,B) or HepG2 cells stably expressing 17K-apo(a) (C,D) were transiently transfected with an expression vector encoding sortilin or the corresponding empty vector (pcDNA). The cells were subjected to a pulse-chase protocol followed by immunoprecipitation of lysate and medium samples using an anti-albumin antibody. Representative fluorographs (A,C) and the results of densitometric analysis (B,D) are shown; the latter are the means ± SEM of 3 independent experiments. No significant differences between pcDNA and sortilin expression plasmid at any time were observed.

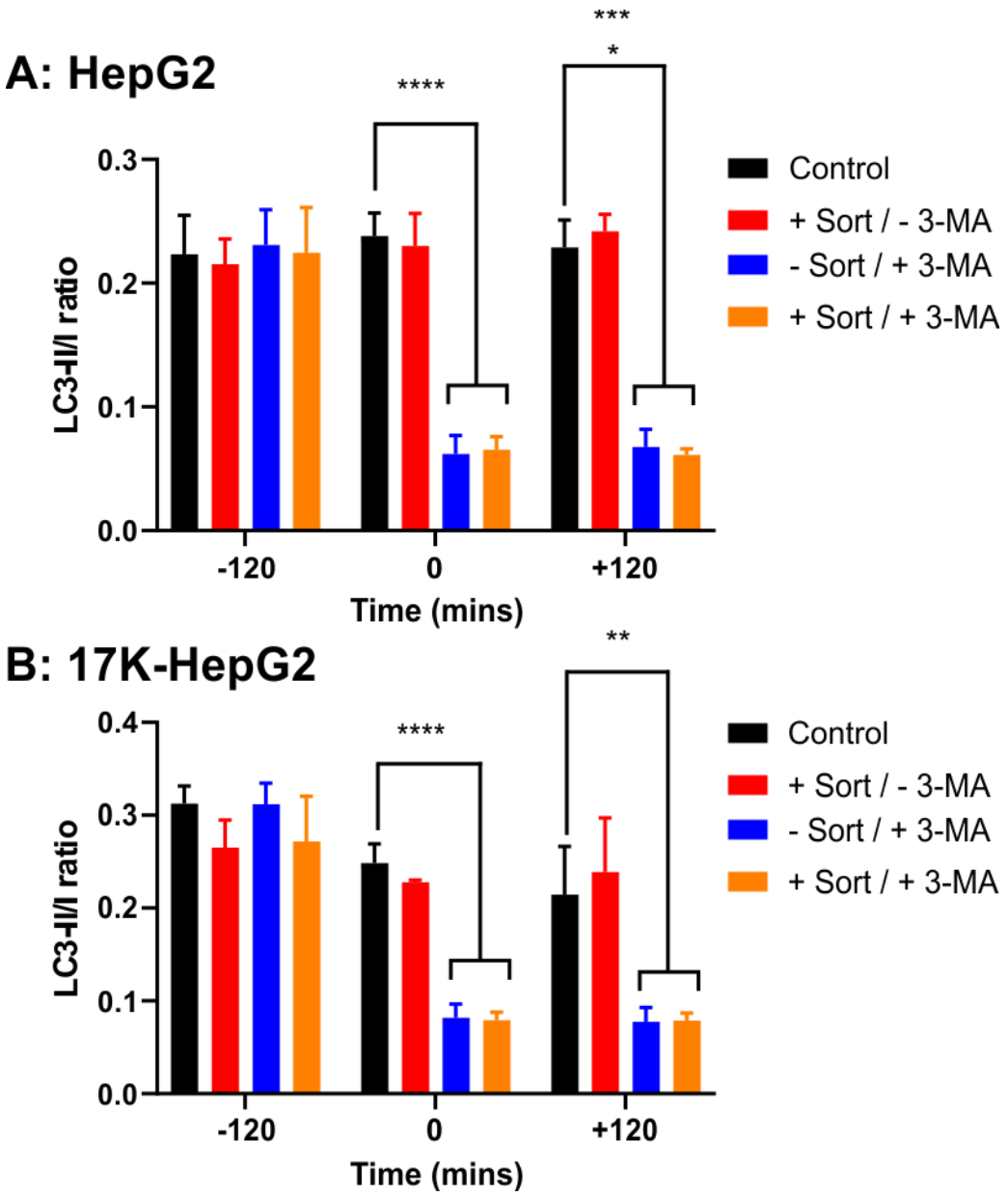

Figure S4. Inhibition of autophagy by treatment with 3-MA. Western blot analysis of LC3 expression in HepG2 (A) and 17K-HepG2 (B) cell lysates incubated in the presence or absence of 3-MA. LC3-I and LC3-II were quantified by densitometric analysis of the blots and a ratio of LC3-II to LC3-I was calculated. The data are the means ± SD of 3 independent experiments. Significance compared to untreated cells was determined by ANOVA and is represented by asterisks (*p<0.05; ** p<0.01; *** p< 0.001; **** p<0.0001).

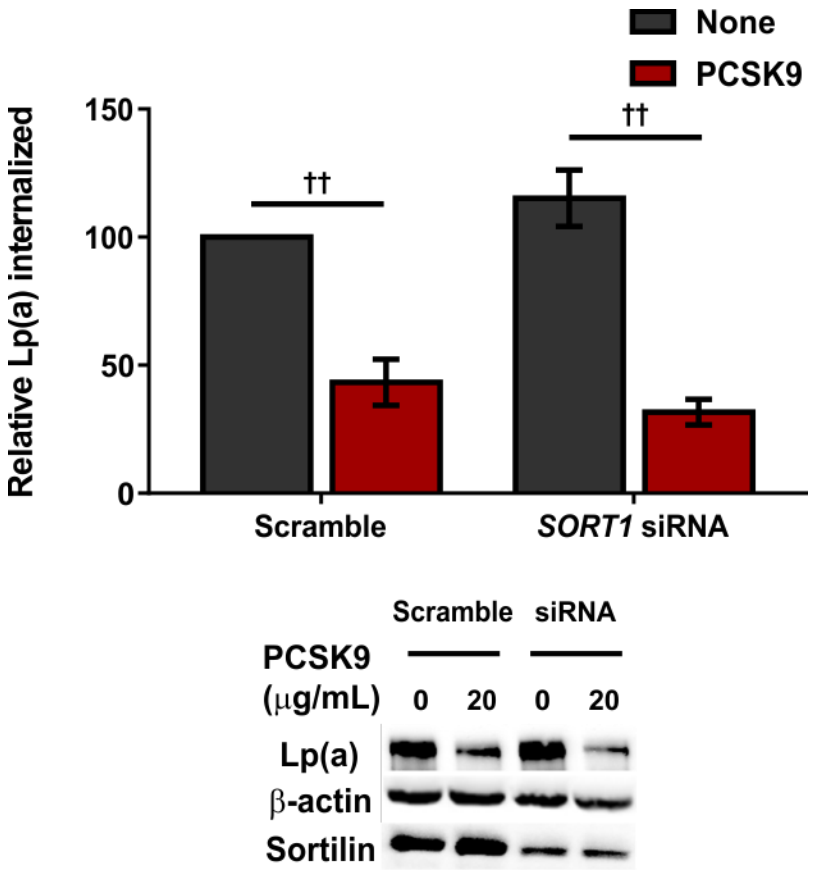

Figure S5. Knockdown of sortilin expression does not alter Lp(a) internalization by HepG2 cells. Cells were transiently transfected with an siRNA against *SORT1* or a scrambled siRNA, as indicated. Cells were grown for 16 hours in LPDS media and subsequently incubated for 4 hours with 10 μg/mL Lp(a). The cells were extensively washed, and lysates were subjected to western blot analysis to determine the amount of Lp(a) internalized. The data shown are normalized using the β-actin signal as an internal control and correspond to the means ± SEM of at least 3 independent experiments. Significance compared to *SORT1* or scrambled siRNA was determined using an unpaired Students' t-test, and is indicated by daggers (††$p<0.01$).

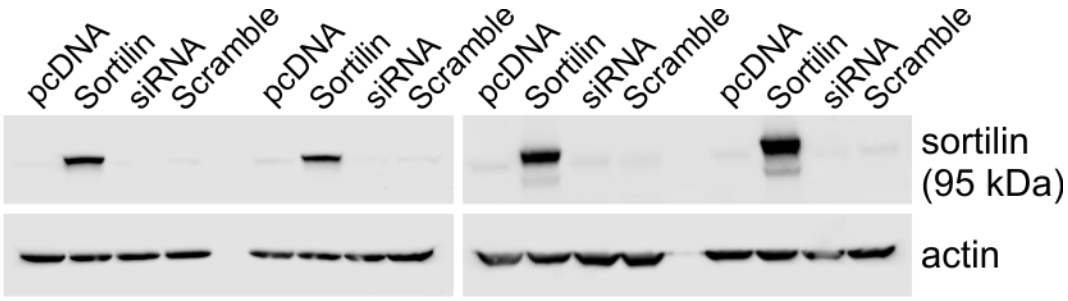

Figure S6. Overexpression and siRNA-mediated knockdown of sortilin. HepG2 cells stably expressing 17K apo(a) were subjected to a pulse-chase protocol to measure the rate of apo(a) secretion. A portion of the lysates collected at the last time point were subjected to western blot analysis with anti-sortilin or anti-actin antibodies. The data from four such experiments are shown and are also representative of expression patterns for Lp(a)/17K apo(a) internalization experiments.