Supplementary data

Cholesteryl ester hydrolase activity is abolished in HSL-/- macrophages but unchanged in macrophages lacking KIAA1363 (Buchebner et al)

Supplementary Table I

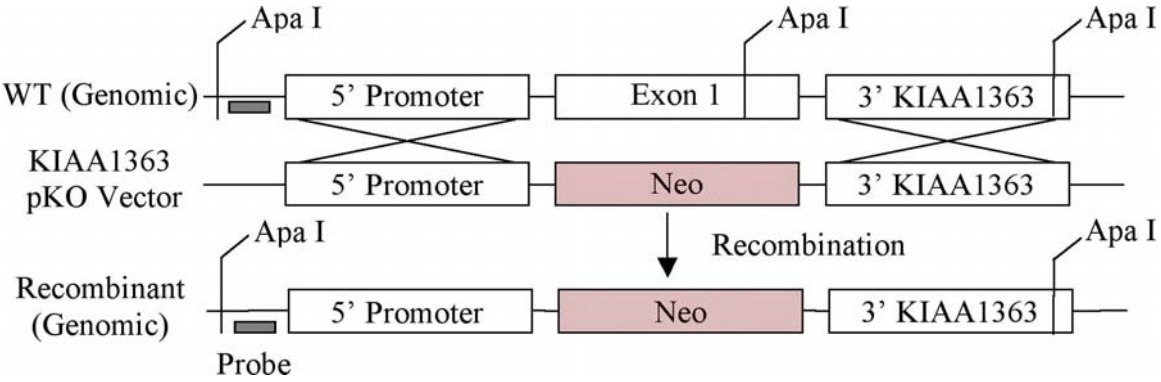
FC and CE concentrations in macrophages of WTD-fed HSL(-/-), KIAA1363(-/-) and wild-type (WT) mice. Mice were fed WTD for 4 weeks. FC and TC concentrations were determined enzymatically, and CE concentrations were calculated. Data are presented as mean values (n=6-7) ± S.E.M.

		µg/mg protein	
	n	FC	CE
macrophages			
WT	7	21.1 ± 0.7	12.1 ± 2.4
HSL(-/-)	6	21.6 ± 1.0	17.9 ± 2.2
KIAA1363(-/-)	6	23.9 ± 1.0	16.4 ± 1.4

Supplementary figures

Figure S1

A



B

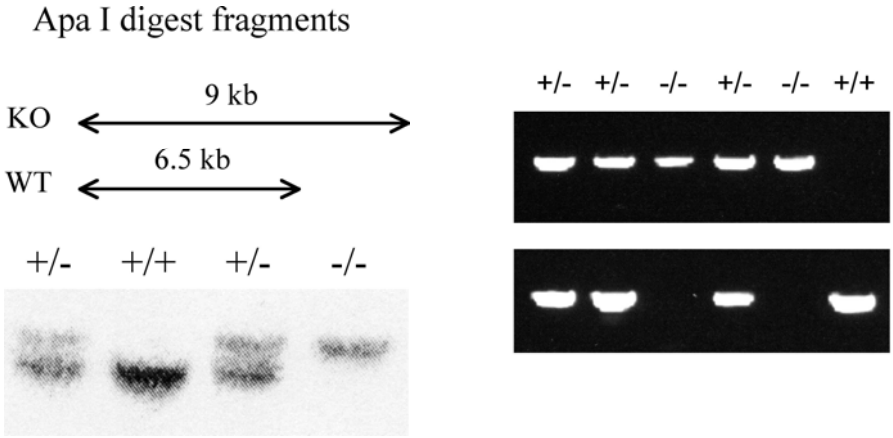


Figure S1: Generation of KIAA1363(-/-) mice. (A) Schematic for generation of KIAA1363(-/-) mice by targeted homologous recombination to remove exon 1 and upstream promoter regions. (B) Confirmation of homologous recombination by Southern blot analysis and PCR.

Figure S2

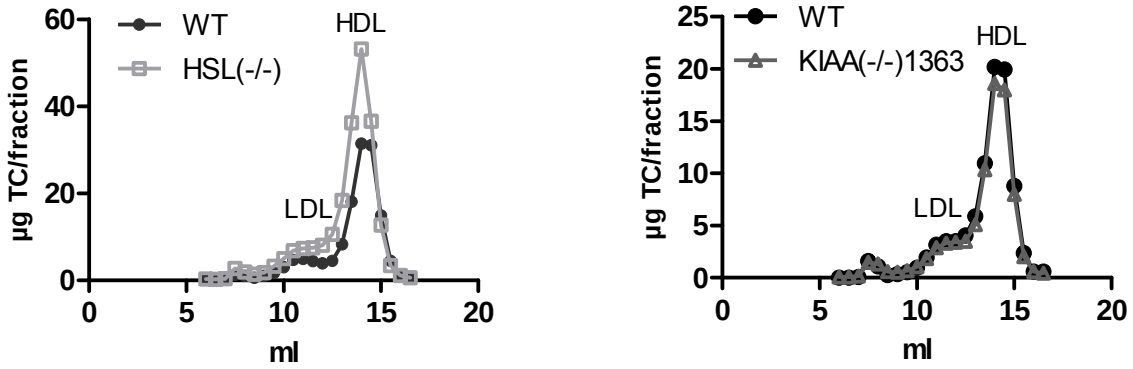


Figure S2: Lipoprotein profiles of WT, HSL(-/-) and KIAA1363(-/-) mice. Plasma pools from overnight fasted WT, HSL(-/-) and KIAA1363 (-/-) were separated using a Pharmacia FPLC system with a Superose 6 column. FPLC fractions were collected and TC concentrations in each fraction were measured enzymatically. Data are presented from a pool of n=4-5 mice.

Figure S3

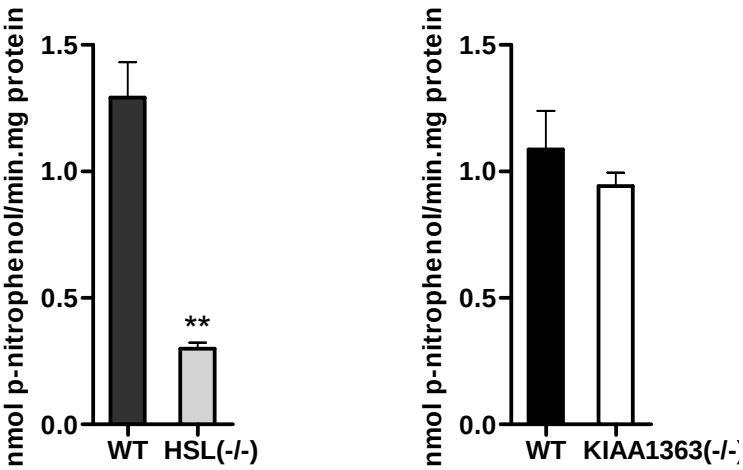


Figure S3: Esterase activity in HSL(-/-) and KIAA1363(-/-) macrophages. Esterase activities were determined in cell lysates of HSL(-/-), KIAA1363(-/-) and WT mice using p-nitrophenylvalerate as substrate. Data are presented as mean values of n=4 performed in duplicate ± SEM. **, $p \leq 0.01$.

Figure S4

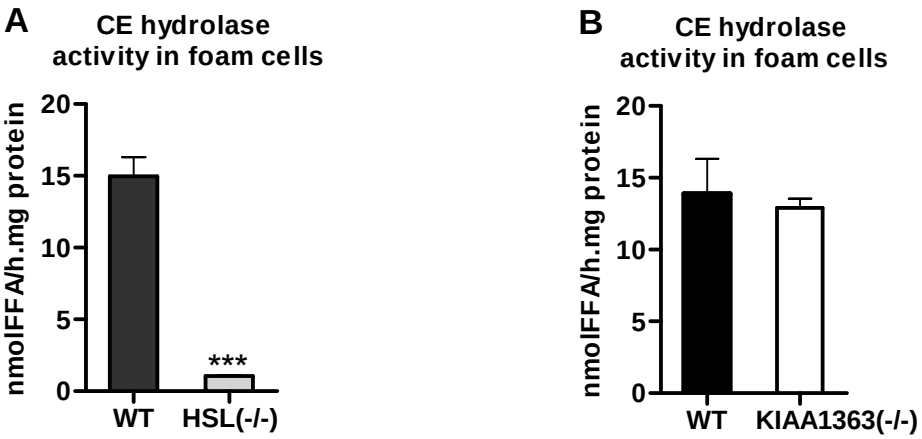


Figure S4: CE hydrolase activities in HSL(-/-) and KIAA1363(-/-)foam cells. CE hydrolase activities were determined in cell lysates of HSL(-/-), KIAA1363(-/-) and WT mice after adding 35.5 µg mixed micelles of PC and PI (3:1, w:w) and 4 µM Na-taurocholate to the substrate. Data are presented as mean values of 3 independent experiments ± SEM (using 3 mice in each experiment). ***, $p \leq 0.001$.

Figure S5

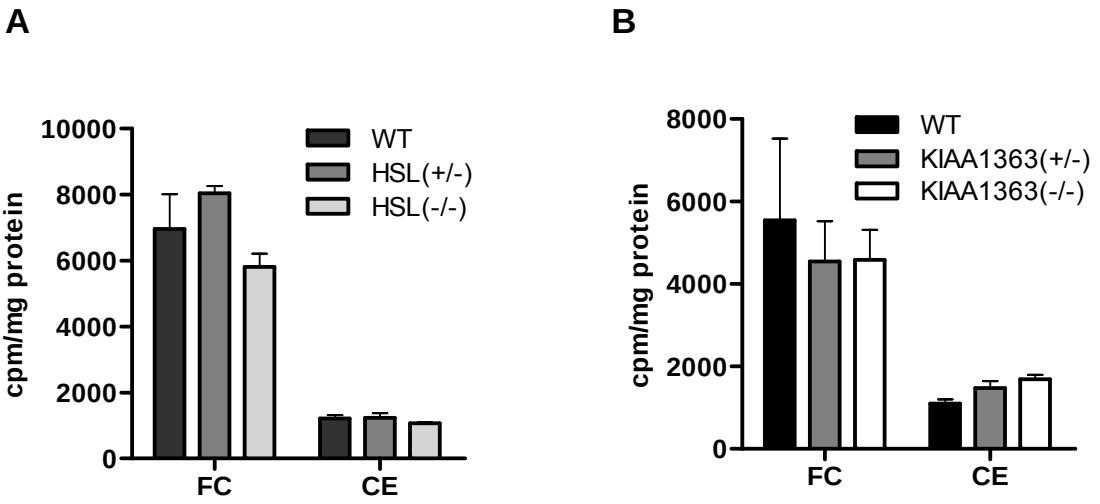


Figure S5: Cholesterol biosynthesis in HSL(-/-) and KIAA1363(-/-) macrophages. MPM of (A) HSL(-/-), HSL(+/-), (B) KIAA1363(+/-) and KIAA1363(-/-) mice as well as WT littermates were incubated with [^{3}H]-acetate for 24h. Lipids were extracted and separated by TLC. Radioactivity in the bands corresponding to FC and CE was determined by liquid scintillation counting and normalized to protein concentration.

Figure S6

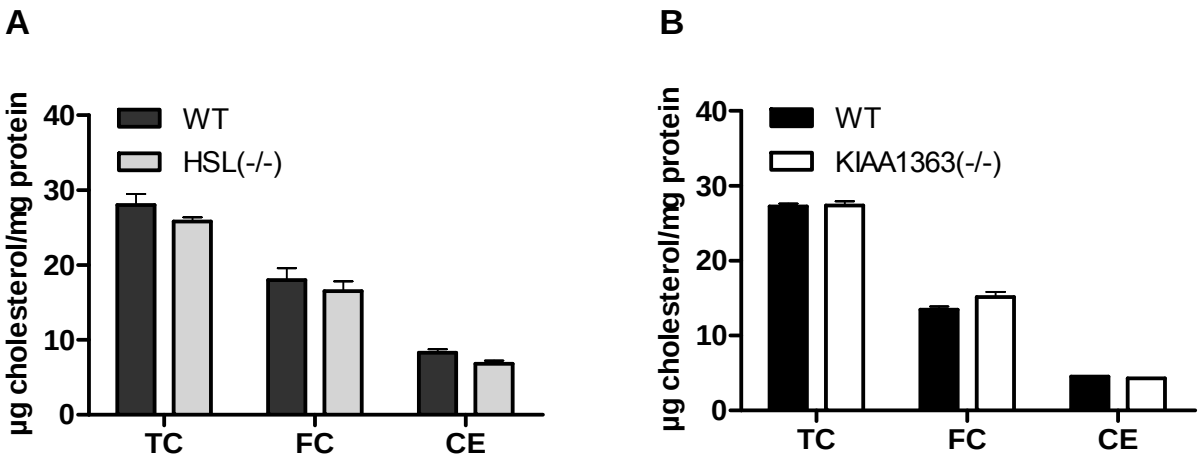


Figure S6: TC, FC and CE content of ß-VLDL loaded (A) HSL(-/-) and (B) KIAA1363(-/-) MPM in comparison to cells from their WT littermates. Cells were loaded with 50 µg/ml [^{3}H]cholesterol-loaded ß-VLDL for 30 h. Lipids were extracted with hexane:isopropanol (3:2, v/v) and 10 µl were assayed by liquid scinitllation counting for TC content. Fourty µl were separated by TLC and bands corresponding to FC and CE were cut out. Radioactivity was measured by liquid scintillation counting. Data are presented as mean values ± SEM of 4 female mice per genotype performed in triplicate.

Figure S7

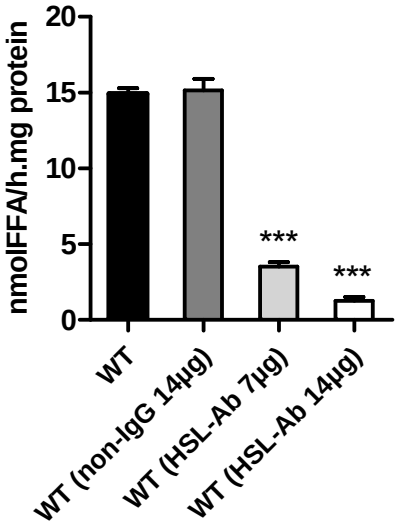


Figure S7: Inhibition of neutral CE hydrolase activity by a specific anti-HSL antibody. Neutral CE hydrolase activities were determined in lysates of WT macrophages by adding 35.5 µg mixed micelles of PC and PI (3:1, w:w) and 4 µM Na-taurocholate to the substrate. The assay was performed in the presence of 7 and 14 µg of a specific anti-HSL antibody targeting the active center of the enzyme. The anti-HSL antibody was made by infecting a rabbit with an adenoviral construct containing the HSL gene. As negative control 14 µg non-IgG was used (isolated from the same rabbit before adenoviral infection). Data are presented as mean values of 4 mice measured in triplicate repeats ± SEM. ***, $p \leq 0.001$.