SUPPLEMENTAL MATERIAL

A Novel Lipid Droplet-Associated Serine Hydrolase Regulates Macrophage Cholesterol Mobilization.

Goo. A novel player in CE turnover from macrophages.

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A

NH2-vlaapqeesnaqk-COOH
NH2-iedvyglngqiehk-COOH
NH2-rdddiikeflpk-COOH
NH2-srfpvwiishagfsvtpk-COOH
NH2-srfpvwiishagfsvtpk-COOH
NH2-fpvwiishagfsvtpk-COOH
NH2-rvpelpvahafllfptier-COOH
NH2-rvpelpvahafllfptier-COOH
NH2-vpelpvahafllfptier-COOH
NH2-vpelpvahafllfptier-COOH
NH2-qlifiipgnpgysafyvpfak-COOH

B

NH2-maseveeqipvreefflcggvetkiikcgpwtnlfekqdvskpk**qlifiipgnpgysafy**
vpfakalytlmk**srfpvwiishagfsvtp**kdkk**vlaapqeesnaqkiedvyglngqiehk**
iaflrahvpkdvklilighsvgtymtlhvmk**rvpelpvahafllfptier**msespngkfatpflcqf
ryllyatsyllfkpcpeviksfiiqklmgqmniklelpltdilqpfclanaaylgsqemvqivk**rdddi**
ikeflpklkfyygktdgwcpvkyyedmkkdfpegniylcekgiphafvldfsqemativaewin
nrpprk-COOH

Figure SI. mLDAH peptides identified by LC-MS/MS. (A) Sequences of the 11 LDAH hits identified in the LD fraction of RAW 264.7 macrophages. Identical peptide sequences are shown in the same color. **(B)** Alignment of the peptides identified by LC-MS/MS with the mLDAH sequence.

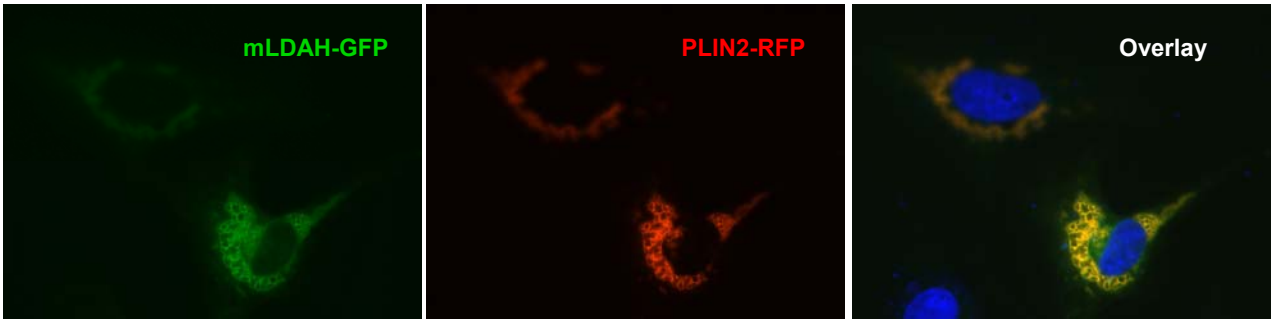

Figure SII. mLDAH-GFP colocalizes with PLIN2 at the LD surface. HeLa cells were co-transfected with mLDAH-GFP (green) and PLIN2-RFP (a LD marker, red) and treated with oleic acid (360 μM) for 18h.

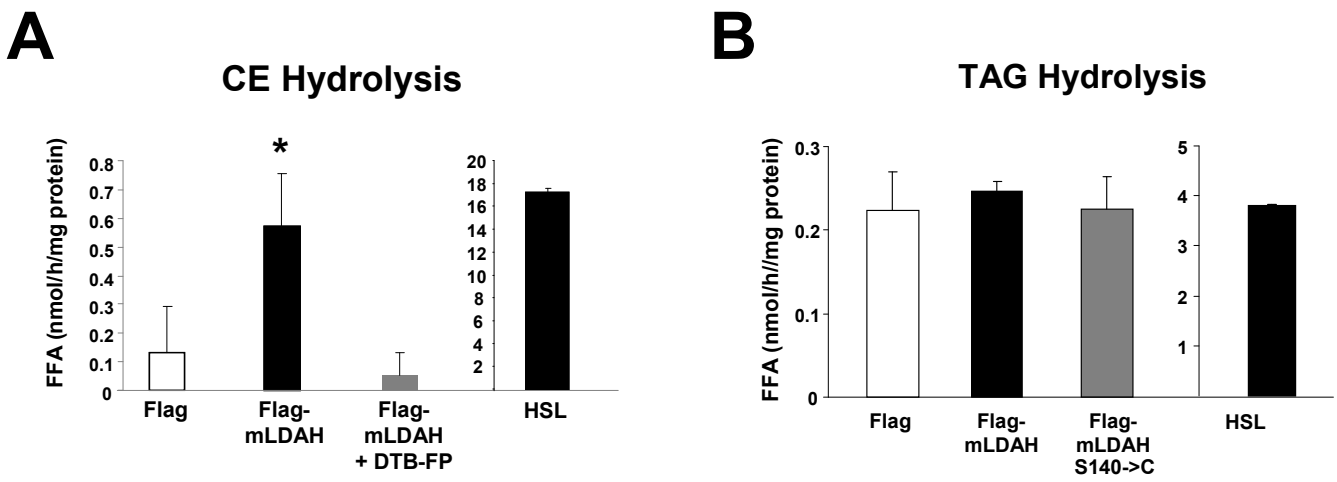

Figure SIII. (A) *In vitro* CE activity assays on protein extracts from HeLa cells transfected with flag- or flag-mLDAH. The activity of the flag-mLDAH lysates was also measured in samples pre-treated with DTB-FP, which acts as a serine hydrolase inhibitor. (n=3). *p<0.05. **(B)** *In vitro* TAG activity assays on protein extracts from HeLa cells transfected with flag-, flag-mLDAH or flag-(S140->C)-mLDAH. (n=3). HSL was used as a positive control for both CE and TAG activities.

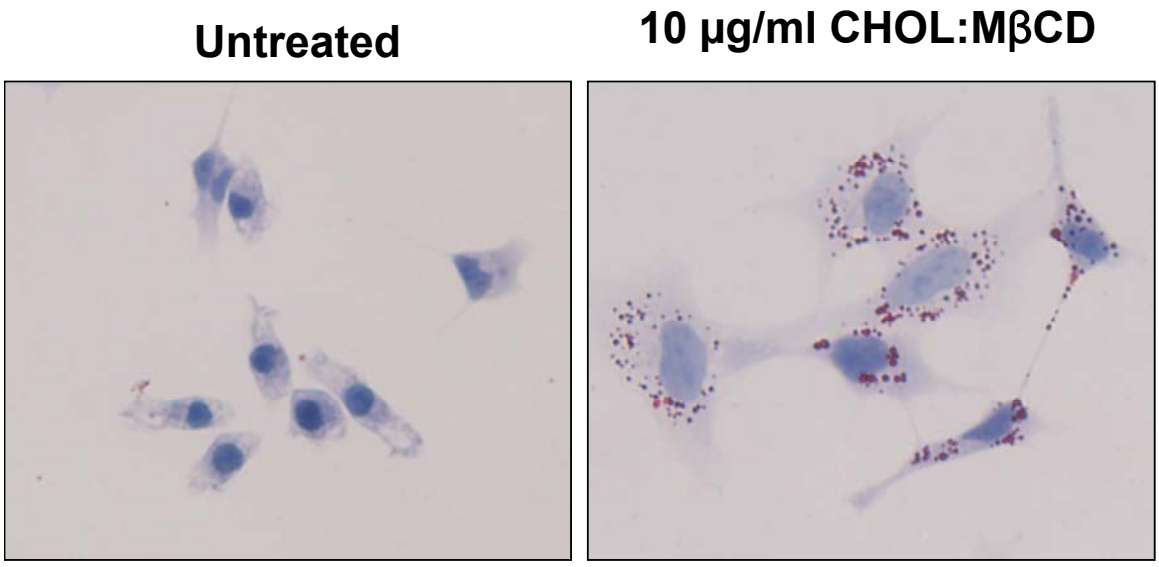

Figure SIV. CHOL:MβCD treatment induces LD formation in HEK293 cells. HEK293 cells remained untreated or were treated with CHOL:MβCD (10 µg/ml). Oil red-O was used to stain neutral lipid (Red). Hematoxylin (blue) was used to stain cell nuclei.

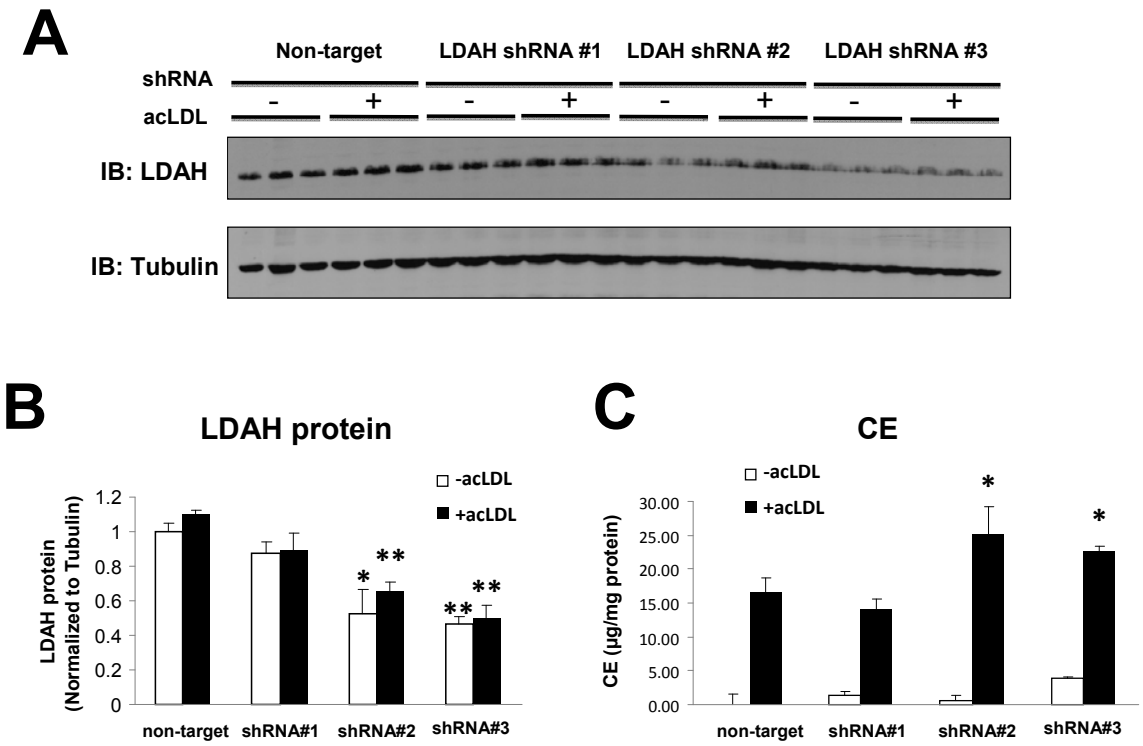

Figure SV. shRNAs that downregulated mLDAH increased CE content in RAW 264.7 macrophages. Three lentiviral shRNAs were tested for mLDAH downregulation in RAW 264.7 macrophages. RAW 264.7 remained untreated (-acLDL) or were treated with acLDL (+acLDL; 50 µg/ml). **(A)** Immunoblots were performed to determine the level of protein downregulation. **(B)** Quantification of the mLDAH bands intensity relative to tubulin. **(C)** Intracellular CE levels. n=3. All data are shown as mean ± SD. *p<0.05,**p<0.005; t-test.

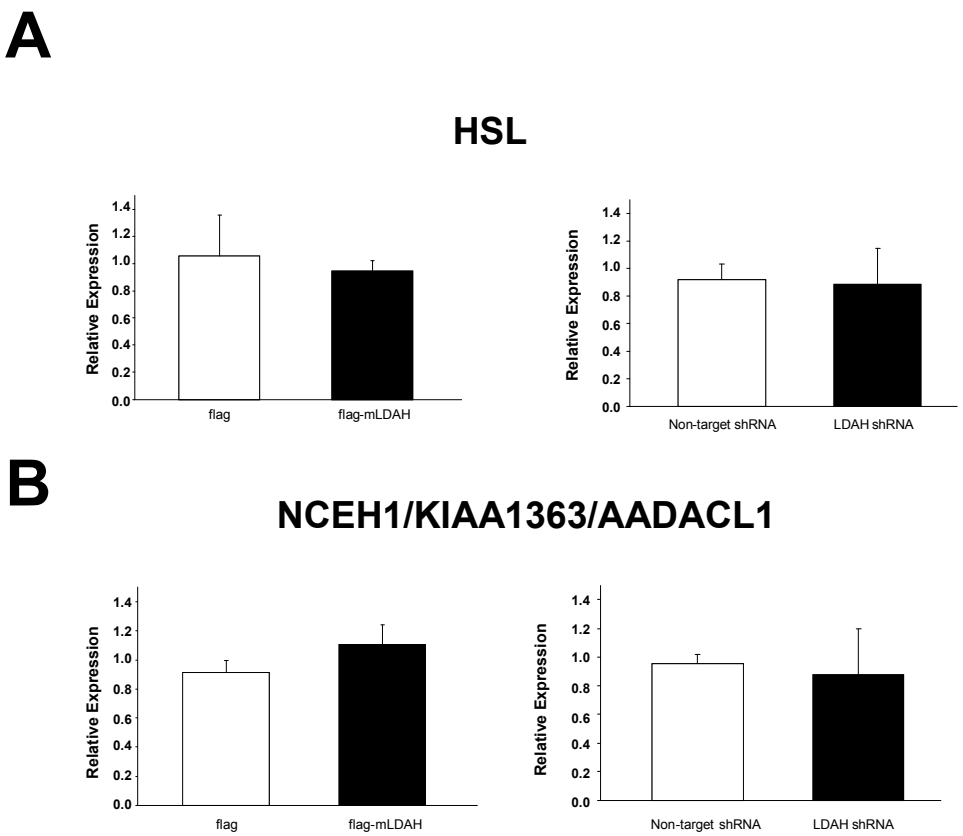

Figure SVI. mLDAH overexpression or downregulation did not change the expression of other candidate CE hydrolases. qPCR analysis of HSL **(A)** and NCEH1/KIAA1363/AADACL1 **(B)** mRNA levels relative to cyclophilin A (CycloA) in Raw 264.7 macrophages transfected with flag-mLDAH or with lentiviral mLDAH shRNA-mediated knockdown. Cells were treated with acLDL (50 μg/ml) for 24h. (n=3). **(C)** CES3/TGH mRNA was undetectable in Raw 264.7 macrophages after 40 cycles of PCR amplification. Liver RNA was used a positive as control for this experiment.

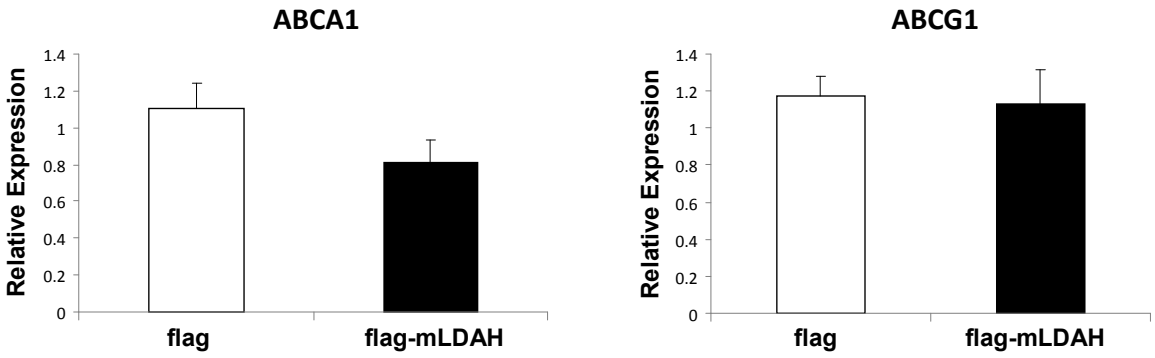

Figure SVII. ABCA1 and ABCG1 mRNA levels were not changed by mLDAH overexpression. Raw 264.7 macrophages were transfected with flag- or flag-mLDAH and incubated for 24 with acLDL (50μg/ml). ABCA1 and ABCG1 mRNA levels were quantified by qPCR and normalized to cyclophilin (n=3).

Table SI. Sequences of primers, siRNAs and shRNAs, used for the studies. The experiments shown in Figure 4B were performed using mLDAH shRNA #3.

Primers	**Sequence**
mLDAH	Forward: 5'-tttcggtgactcccaaagac-3' Reverse: 5'-cttcatcacgtgaagggtca-3'
LCM-mLDAH	Forward: 5'-ctggtgtctgcacaaggatg-3' Reverse: 5'-gattcccaccagtgaccaag-3'
ABCA1	Forward: 5'-ctccaggattttctggtgga-3' Reverse: 5'-cacagagggcagaaacttcc-3'
ABCG1	Forward: 5'-tccatcgtctgtaccatcca-3' Reverse: 5'-tactcccctgatgccacttc-3'
HSL	Forward: 5'-atgtgcacttctggaaagcct-3' Reverse: 5'-agcaggcggcttaccctcaca-3'
NCEH1/ KIAA1363/ AADACL1	Forward: 5'-tctccgaaaccagaagagcc-3' Reverse: 5'-tgtgcacagctggtcatagt-3'
CES3/TGH	Forward: 5'-agatcactgcaggggacaaa-3' Reverse: 5'-gatcccaaggcgatactgga-3'
Cyclophilin A	Forward: 5'-tttgggaaggtgaaagaagg-3' Reverse: 5'-ttacaggacattgcgagcag-3'
siRNAs	
hLDAH	Sense: GGACAUUUAUGGACUAAAUtt Antisense: AUUUAGUCCAUAAAUGUCCtt
shRNAs	
mLDAH shRNA #1	Sense: CCGUGUCCUGAAGUCAUAA Antisense : UUAUGACUUCAGGACACGG
mLHAH shRNA #2	Sense: UCGAUACCUGCUCUAUGCU Antisense : AGCAUAGAGCAGGUAUCGA
mLDAH shRNA #3	**Sense: CUCAGAAGUCGAGGAACAA** **Antisense : UUGUUCCUCGACUUCUGAG**

Table SII. LD associated proteins identified by LC-MS/MS. The references indicate proteins found in other LD proteomic analyses in mammalian cells.

Fragment	Protein Name	Peptides	GI	MW (kDa)	References
1	BIP	9	2598562	72.5	[1-9]
	Unnamed	4	74147026	83.5	
	Plastin-2	3	31543113	70.7	
	UBXN4	3	30913398	56.8	[8]
2	Vimentin	12	2078001	51.6	[1, 2]
	PLPL2 (ATGL)	8	81896337	54.5	[2, 4, 8]
	Ubiquitin B	8	18044723	34.4	[4]
	Prolyl-4-Hydrolase	7	42415475	57.4	[2-4]
	CAP-1	6	729032	51.9	
	Unnamed	6	12852157	58.8	
	Pyruvate kinase M	4	551295	58.4	[2]
	Alpha-tubulin 8	3	8394493	50.7	
	PLCα	3	200397	57.0	[2]
3	Perilipin 2 (ADFP)	48	116235489	46.9	[1-8, 10-12]
	Enolase 1alpha	7	70794816	47.5	[2]
	Protein disulfide-isomerase A3	5	112293264	57.1	[5-7]
	Interferon gamma induced GTPase	4	28261389	48.8	
	LysoPC acyltransferase 1	3	148747363	60.4	[8]
4	Gamma-actin	29	809561	41.3	
	Ancient ubiquitous protein	12	90403601	49.7	[1, 3, 4, 8]
	Unnamed	6	74144652	38.9	
	VAT-1	6	33859662	43.3	[3-5, 12]
	ABHD5 (CGI-58)	5	13385690	39.5	[1, 2, 4, 6, 8, 12]
	IRGM-1	3	6680351	47.1	[12]
5	**RIKEN cDNA 1110057K04 gene (LDAH)**	**11**	**55777092**	**37.7**	
	Unnamed	7	74142813	50.5	
	Annexin A1	5	124517663	39.0	[12]
	Annexin A2	4	6996913	38.9	[2, 4, 10, 12]
	Unnamed	4	74181454	50.8	
	Ribosomal protein, large, P0	3	13277927	34.3	[2]
6	RAB7	13	1050551	23.8	[1-8]
	DHRS-1	9	31980844	34.5	[2, 4, 8]
	Diaphorase-1	8	19745150	34.3	[1-4, 6-8, 10]
	RAB2A	7	10946940	23.7	[2, 7, 8, 12]
	RAB11B	6	6679583	24.6	[7, 8, 12]
	RAB14	6	63087697	22.1	[1, 2, 4, 7]
	RAB5C	5	113866024	23.6	[1-3, 7, 10, 12]
	HSP70	5	309319	71	[1, 2, 4, 6, 9, 11]
	RAB18	4	30841008	23.3	[1-4, 6-8, 12]
	RAP1B	3	7661678	21.0	[8, 10, 12]

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