SUPPLEMENTARY INFORMATION

to:

Systematic RNA-interference in primary human monocyte-derived macrophages: A high-throughput platform to study foam cell formation

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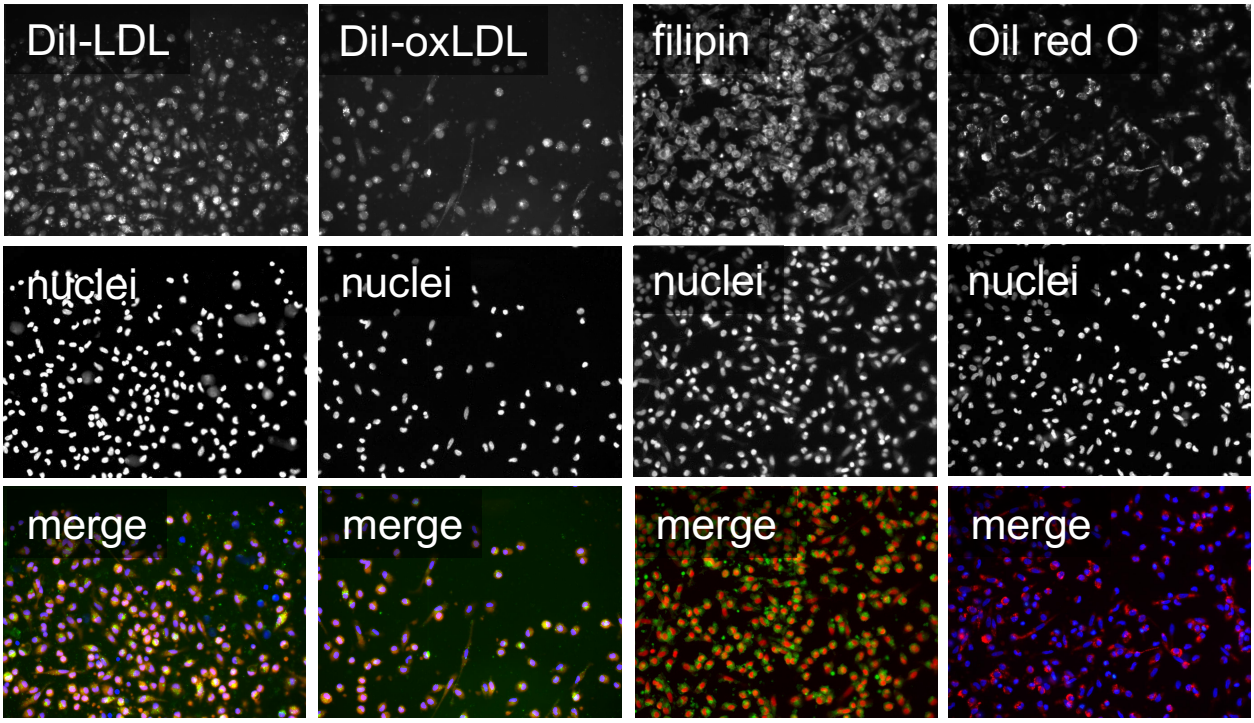
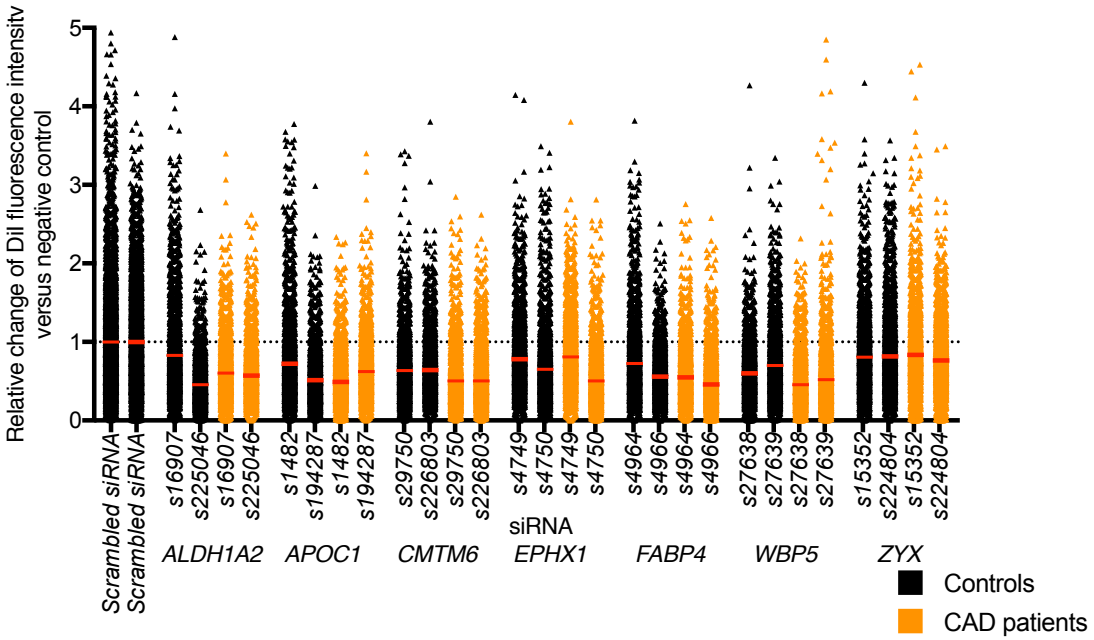
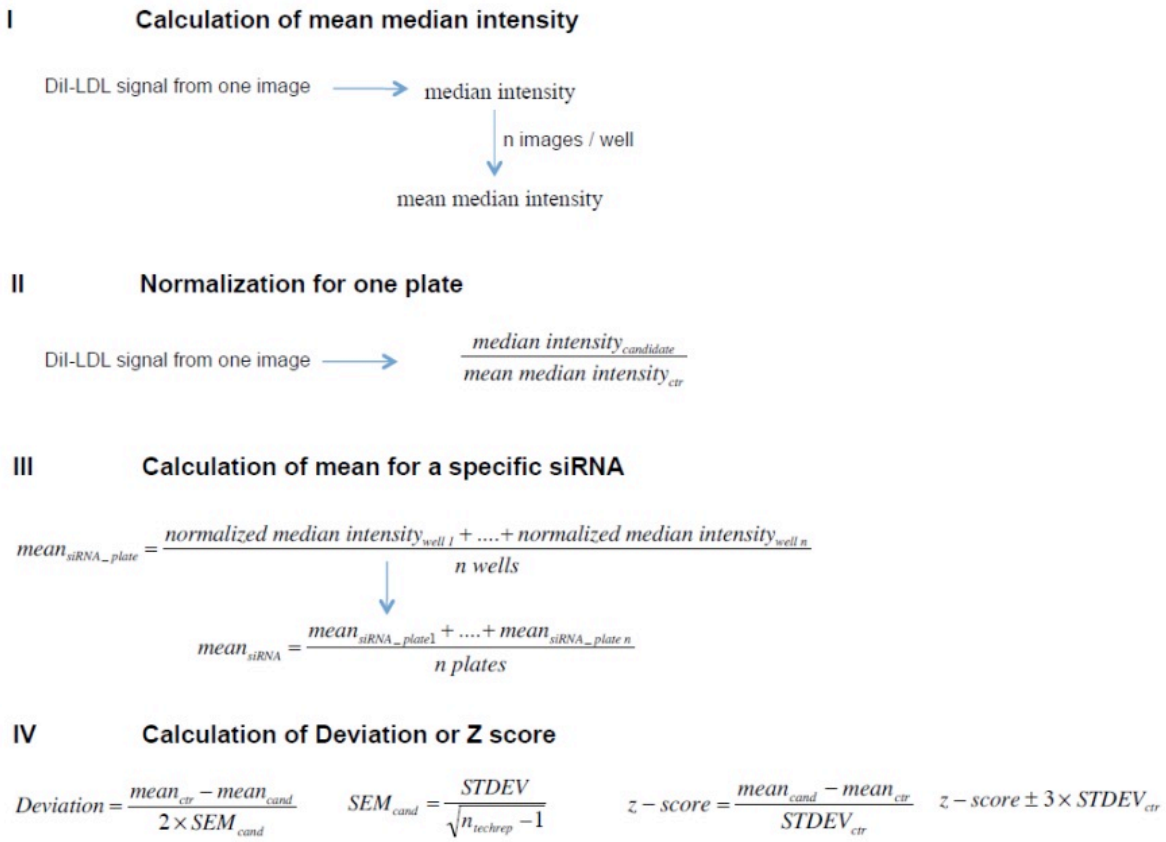


Supplemental Figure I. Automated microscopy to study lipid metabolism in cultured primary human monocyte-derived macrophages. Representative widefield microscopic images acquired on an automated high-throughput microscopy platform of macrophages analyzed for cellular uptake of DiI-LDL (left column), DiI-oxLDL (2nd column), cellular cholesterol content using filipin (3rd column), or lipid droplets using Oil-red O (right column).



Supplemental Figure II. Relative change in DiI-LDL fluorescence intensities for seven candidate genes relative to control siRNA-treated cells. Each dot indicates the mean DiI fluorescence intensity from cell masks of one individual image frame. For each candidate gene nominated as putative foam-cell regulator during the primary screen, two siRNAs were spotted each on three wells of a 384 well plate. Shown are results from cells of 10 healthy individuals (black) and 10 patients with coronary artery disease (orange) as obtained during the replication screen with 3 technical replicates per individual. Intensity values were normalized to the mean of all negative controls wells.



Supplemental Figure III. Visualization of Data Analysis Strategy. For details, see Methods.

SUPPLEMENTAL TABLES

Table S1

Gene	Ensembl ID	Hagg et al. [ref. 13]		Cho et al. [ref. 14]		other reference
		healthy	CAD	MCSF/oxLDL	CXCL4/oxLDL	
ACTR3	ENSG00000115091		x			
PLIN2	ENSG00000147872	x	x	x	x	
AKR1B1	ENSG00000085662	x			x	
ALDH1A2	ENSG00000128918				x	
ANXA1	ENSG00000135046	x			x	
APOC1	ENSG00000130208	x	x	x	x	
APOL2	ENSG00000128335				x	
AQP9	ENSG00000103569	x			x	
ATP6V0E	ENSG00000113732		x	x	x	
BACH1	ENSG00000156273				x	
BASP1	ENSG00000176788					x
BATF	ENSG00000156127		x	x	x	
BIRC3	ENSG00000023445		x	x	x	
BSCL2	ENSG00000168000		x	x	x	
C14orf1	ENSG00000133935	x	x	x	x	
CALR	ENSG00000179218		x	x	x	
CAP2	ENSG00000112186	x				
CCL1	ENSG00000108702		x	x	x	
CCL22	ENSG00000102962	x			x	
CCPG1	ENSG00000214882		x	x	x	
CDKN2B	ENSG00000147883					x
CMTM6	ENSG00000091317	x				
CLIC1	ENSG00000213719					x
CORO1A	ENSG00000102879	x	x	x	x	
COX5B	ENSG00000135940		x	x	x	
CRABP2	ENSG00000143320	x	x	x	x	
CRIP1	ENSG00000213145	x	x	x	x	
DBI	ENSG00000155368	x				
DHRS9	ENSG00000073737		x	x	x	
DPP7	ENSG00000176978	x				
DUSP4	ENSG00000120875				x	
EPHX1	ENSG00000143819	x	x	x	x	
FABP4	ENSG00000170323		x	x	x	
FADS1	ENSG00000149485	x	x	x	x	
FADS2	ENSG00000134824	x	x	x	x	
FASN	ENSG00000169710		x	x	x	
SLC48A1	ENSG00000211584					x
GLT25D1	ENSG00000130309					x
IGFLR1	ENSG00000126246				x	x
GDF15	ENSG00000130513		x	x	x	
GLRX	ENSG00000173221	x			x	
GPX4	ENSG00000167468				x	
H3F3A	ENSG00000163041		x	x	x	
HEBP1	ENSG00000013583	x			x	
HMOX1	ENSG00000100292		x	x	x	
HNRPM	ENSG00000099783				x	
HPCAL1	ENSG00000115756	x			x	
KRR1	ENSG00000111615	x			x	
ACOT13	ENSG00000112304					x
RNF19B	ENSG00000116514					x
ID2	ENSG00000115738	x				

IFITM1	ENSG00000185885		x	x	x	
ZNF706	ENSG00000120963					
LPL	ENSG00000175445	x			x	
LRMP	ENSG00000118308				x	
TMEM97	ENSG00000109084	x	x	x	x	
MGLL	ENSG00000074416	x				
MORF4L1	ENSG00000185787					x
MRPS2	ENSG00000122140					x
MYB	ENSG00000118513		x	x	x	
IL32	ENSG00000008517		x	x	x	
PLAUR	ENSG00000011422	x				
PLEK	ENSG00000115956	x				
PPAP2B	ENSG00000162407	x			x	
PSIP1	ENSG00000164985	x			x	
RAP2B	ENSG00000181467				x	
C13orf15	ENSG00000102760					x
RRAGD	ENSG00000025039				x	
S100A6	ENSG00000197956	x				
SAT1	ENSG00000130066				x	
SCD	ENSG00000099194	x			x	
SH2D1A	ENSG00000183918				x	
SMARCA4	ENSG00000127616					x
SMPDL3A	ENSG00000172594		x	x		
SNF8	ENSG00000159210					x
SQLE	ENSG00000104549	x	x	x	x	
SQSTM1	ENSG00000161011	x	x	x	x	
TALDO1	ENSG00000177156	x			x	
TNFRSF21	ENSG00000146072		x	x	x	
TPT1	ENSG00000133112		x	x	x	
TXN	ENSG00000136810	x	x	x	x	
GLRX3	ENSG00000108010		x	x	x	
UCHL1	ENSG00000154277		x	x	x	
VAT1	ENSG00000108828				x	
WBP5	ENSG00000185222		x	x	x	
ZBED2	ENSG00000177494				x	
ZNF259	ENSG00000109917					x
ZYX	ENSG00000159840	x				
LGALS3BP	ENSG00000108679					x

Supplemental Table I. 89 genes regulated during foam cell formation functionally analyzed for an impact on cellular DiI-LDL uptake during this study. Genes were selected based on their differential mRNA expression based on references [10,13].

Table S2

Parameter	Total	Both screens combined			Primary screen			Replication Screen		
		Control	CAD	p	Control	CAD	p	Control	CAD	p
n	**32**	16	16		6	6		10	10	
Age (years)	**60±9**	56±7	65±9	0.003	55±8	63±10	0.240	56±7	66±8	0.007
CAD	**16 (50%)**	-	-	-	-	-	-	-	-	-
Hypertension	**19 (59%)**	4 (25%)	11 (69%)	<0.001	2 (33%)	5 (84%)	0.102	2 (20%)	10 (100%)	<0.001
Hyperlipidemia	**19 (59%)**	8 (50%)	11 (69%)	0.134	5 (83%)	5 (84%)	0.102	3 (30%)	6 (60%)	0.527
Diabetes mellitus	**16 (50%)**	2 (13%)	3 (19%)	0.012	0 (0%)	2 (33%)	0.414	2 (20%)	1 (10%)	0.011
Family history	**14 (44%)**	5 (31%)	9 (56%)	0.617	3 (50%)	3 (50%)	1.000	2 (20%)	6 (60%)	0.527
Smoking history	**16 (50%)**	9 (56%)	7 (44%)	0.617	1 (17%)	5 (84%)	0.102	8 (80%)	2 (20%)	0.058
MI	**3 (9%)**	0 (0%)	3 (19%)	0.012	0 (0%)	3 (50%)	<0.001	0 (0%)	0 (0%)	1.000
PCI	**15 (47%)**	0 (0%)	15 (94%)	<0.001	0 (0%)	6 (100%)	<0.001	0 (0%)	9 (90%)	0.011
CABG	**3 (9%)**	0 (0%)	3 (19%)	0.012	0 (0%)	1 (17%)	0.102	0 (0%)	2 (20%)	0.058
Peripheral artery disease	**2 (6%)**	0 (0%)	2 (13%)	0.003	0 (0%)	1 (17%)	0.102	0 (0%)	1 (10%)	0.011
Stroke, TIA	**1 (3%)**	0 (0%)	1 (6%)	<0.001	0 (0%)	1 (17%)	0.102	0 (0%)	0 (0%)	1.000
Total cholesterol (mg/dl)	**183±42**	195±49	171±29	0.138	147±23	164±23	0.240	224±34	175±33	0.005
HDL (mg/dl)	**44±15**	44±15	44±16	0.809	34±16	40±21	0.310	50±11	47±14	0.353
LDL (mg/dl)	**109±29**	121±30	98±25	0.047	94±13	95±11	0.937	138±25	99±31	0.011
Triglycerides (mg/dl)	**149±74**	154±77	144±73	0.809	97±45	138±87	1000	188±73	148±69	0.247

Supplemental Table II. Clinical and demographic characteristics of patients analyzed in this study. Control individuals and coronary artery disease (CAD) patients included in primary and replication screens are shown. Means ± standard deviations or absolute numbers (percentages) are indicated. P values were calculated by Mann-Whitney test (continuous variables) or Chi square test (categorical variables). N, sample number; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; TIA, transient ischemic attack; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SUPPLEMENTAL DATASHEETS

see accompanying file: [Domschke_SupplDatasheets]

Supplemental Datasheet 1
Results of primary RNAi-screening for functional regulators of DiI-LDL uptake into cultured primary human macrophages among 89 foam-cell regulated genes
The impact on DiI-LDL uptake upon candidate gene knockdown was analyzed with 3 independent siRNAs/gene. SiRNAs meeting significance criteria (z-score/Deviation >|1|) are highlighted in red (increased LDL-uptake relative to control-siRNA treated cells) or green (reduced LDL-uptake relative to control-siRNA treated cells) as mean medians of deviations (Md). Negative values indicate reduction in DiI-LDL uptake. C, control individuals; P, coronary-artery disease patients. Column V [Md(C+P)] indicates means across patients and control individuals in this study. Column W [Md(C)-Md(P)] indicates difference in means between patients and controls in this study. *** met criteria for inclusion in validation screening; NA, not available.

Supplementary Datasheet 2
Results of validation RNAi-screening for functional regulators of DiI-LDL uptake into cultured primary human macrophages among 7 putative foam-cell regulators identified in primary screen
For validation of primary screen results, the impact on DiI-LDL uptake upon knockdown of seven selected candidate genes was analyzed with 2 independent siRNAs/gene. SiRNAs meeting significance criteria (z-score/Deviation >|1|) are highlighted in green (reduced LDL-uptake relative to control-siRNA treated cells) as mean medians of deviations (Md). Negative values indicate reduction in DiI-LDL uptake. C, control individuals; P, coronary-artery disease patients. Columns AE and AI [Md(C+P)] indicate means across patients and control individuals in this study. Columns AF and AJ [Md(C)-Md(P)] indicate difference in means between patients and controls in this study. siRNAs with Md(C)-Md(P) >|1| are highligthed in red. Columns AG-AJ indicate meas for all measurements per siRNA during replication screen. Genes with ³2 significant siRNAs validated in replication screen are highlighted by **.; NA, not available.