SUPPLEMENTAL MATERIALS

Anti-inflammatory effects of HDL in macrophages predominate over pro-inflammatory effects in atherosclerotic plaques

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Supplemental Tables and Figures

Supplemental Table I: Top 20 GO categories associated with rHDL-induced genes

GO biological process complete	# Genes	Fold Enrichment	Bonferroni-adjusted P
cholesterol biosynthetic process (GO:0006695)	14	13.22	7.43E-08
chemokine-mediated signaling pathway (GO:0070098)	11	5.93	3.37E-02
macroautophagy (GO:0016236)	17	5.09	7.58E-04
vacuole organization (GO:0007033)	19	4.42	1.17E-03
regulation of GTPase activity (GO:0043087)	28	2.87	9.99E-03
carboxylic acid biosynthetic process (GO:0046394)	25	2.84	4.36E-02
phospholipid metabolic process (GO:0006644)	27	2.82	2.06E-02
carbohydrate derivative biosynthetic process (GO:1901137)	43	2.67	1.06E-04
organophosphate biosynthetic process (GO:0090407)	33	2.5	2.19E-02
regulation of protein kinase activity (GO:0045859)	47	2.16	9.59E-03
oxidation-reduction process (GO:0055114)	60	2.1	8.75E-04
organonitrogen compound biosynthetic process (GO:1901566)	72	1.98	4.54E-04
chemical homeostasis (GO:0048878)	66	1.85	1.54E-02
positive regulation of catalytic activity (GO:0043085)	64	1.85	2.26E-02
regulation of intracellular signal transduction (GO:1902531)	97	1.74	9.17E-04
positive regulation of cellular protein metabolic process (GO:0032270)	84	1.68	2.39E-02
regulation of cellular component organization (GO:0051128)	135	1.64	6.39E-05
organic substance transport (GO:0071702)	97	1.63	1.44E-02
cellular localization (GO:0051641)	106	1.61	8.52E-03
regulation of localization (GO:0032879)	136	1.48	1.72E-02

Supplemental Table II: Top 20 GO categories associated with rHDL-repressed genes

GO biological process complete	# Genes	Fold Enrichment	Bonferroni-adjusted P
cytokine biosynthetic process (GO:0042089)	7	15.3	4.52E-03
cellular response to interferon-beta (GO:0035458)	14	11.77	3.19E-07
microglial cell activation (GO:0001774)	7	10.93	4.07E-02
phospholipid transport (GO:0015914)	13	7.89	1.75E-04
regulation of interleukin-1 beta production (GO:0032651)	10	6.56	3.74E-02
defense response to virus (GO:0051607)	29	6.09	2.76E-10
positive regulation of tumor necrosis factor production (GO:0032760)	14	5.88	1.77E-03
positive regulation of leukocyte migration (GO:0002687)	23	5.63	5.80E-07
positive regulation of interleukin-6 production (GO:0032755)	13	5.26	1.62E-02
negative regulation of T cell activation (GO:0050868)	17	5.16	5.87E-04
positive regulation of leukocyte chemotaxis (GO:0002690)	13	4.96	3.07E-02
leukocyte migration (GO:0050900)	25	4.41	1.26E-05
leukocyte chemotaxis (GO:0030595)	15	4.2	4.06E-02
positive regulation of lymphocyte proliferation (GO:0050671)	17	4.13	1.23E-02
positive regulation of innate immune response (GO:0045089)	19	4.02	4.49E-03
regulation of lymphocyte differentiation (GO:0045619)	19	3.8	1.02E-02
regulation of T cell proliferation (GO:0042129)	19	3.8	1.02E-02
regulation of cytokine secretion (GO:0050707)	20	3.64	1.01E-02
positive regulation of cytokine production (GO:0001819)	39	3.31	1.49E-06
response to lipopolysaccharide (GO:0032496)	23	3.2	1.48E-02

Supplemental Table III: GO categories associated with rHDL-induced and rHDL-repressed genes in cholesterol loaded macrophages

GO biological process complete for rHDL-induced genes	# Genes	Fold Enrichment	Bonferroni-adjusted P
cholesterol biosynthetic process (GO:0006695)	5	> 100	2.29E-12
oxidation-reduction process (GO:0055114)	7	12.04	5.53E-03
GO biological process complete for rHDL-repressed genes	**# Genes**	**Fold Enrichment**	**Bonferroni-adjusted P**
response to endoplasmic reticulum stress (GO:0034976)	7	23.87	1.33E-04
protein folding (GO:0006457)	5	21.54	2.99E-02

Supplemental Table IV: rHDL-induced cholesterol biosynthetic genes in cholesterol loaded and non-cholesterol loaded macrophages

Non-cholesterol loaded macrophages	Cholesterol loaded macrophages
Dhcr24	Dhcr24
Hmgcr	Sc5d
Nsdhl	Fdft1
Insig1	Cyp51
Sc5d	Hsd17b7
Cyb5r3	
Tm7sf2	
Lss	
Dhcr7	
Fdft1	
Cyp51	
Fdps	
Hsd17b7	
Pmvk	

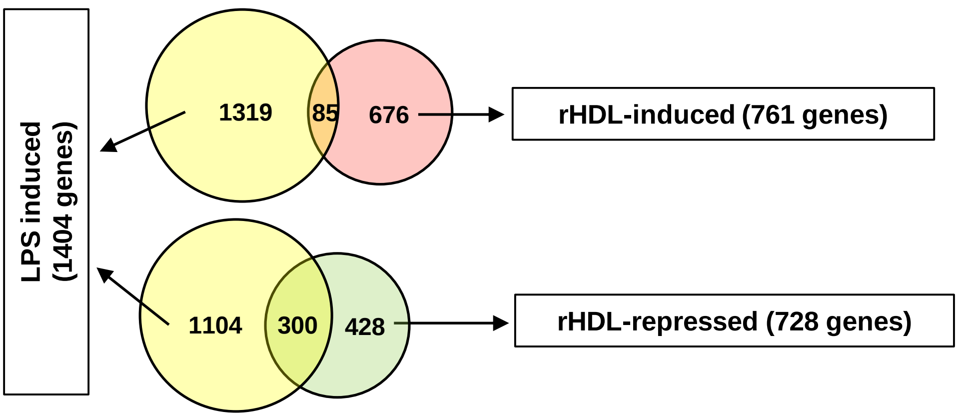
Supplemental Table V: List of primers

Gene	Forward sequence	Reverse sequence
<u>Mouse</u>		
M36b4	CCTGAAGTGCTCGACATCAC	CCACAGACAATGCCAGGAC
Tnfa	CCAGACCCTCACACTCAGATC or CCCTCACACTCAGATCATCTTCT	CACTTGGTGGTTTGCTACGAC or GCTACGACGTGGGCTACAG
Ccl2	CCCAATGAGTAGGCTGGAGA or TTAAAAACCTGGATCGGAACCAA	TCTGGACCCATTCCTTCTTG or GCATTAGCTTCAGATTTACGGGT
Cxcl1	CCCAAACCGAAGTCATAGCC or TGGCTGGGATTCACCTCAAG	TGGGGACACCTTTTAGCATC or CCGTTACTTGGGGACACCTT
Cxcl2	AGTGAACTGCGCTGTCAATG	TTAGCCTTGCCTTTGTTCAG
Il1b	TGTGAATGCCACCTTTTGACA or GGGCTGCTTCCAAACCTTTG	GGTCAAAGGTTTGGAAGCAG or TGATACTGCCTGCCTGAAGCTC
Il6	ACAACCACGGCCTTCCCTACTT	CACGATTTCCCAGAGAACATGTG
Ifit3	AGTGAGGTCAACCGGGAATCT	TCTAGGTGCTTTATGTAGGCCA
Mx1	AAACCTGATCCGACTTCACTTCC	TGATCGTCTTCAAGGTTTCCTTGT
Ifnβ	TGAACTCCACCAGCAGACAG	AAGATCTCTGCTCGGACCAC
Oasl1	CCAGGAAGAAGCCAAGCACCATC	AGGTTACTGAGCCCAAGGTCCATC
Abca1	CAGCTTCCATCCTCCTTGTC	CCACATCCACAACTGTCTGG
Hmgcr	TTTCTGGCGCTTTCAGAGAC or GACTGTGGTTTGTGAAGCCG	TTAACCCACGGAGAGGTGAG or GTTGTAGCCGCCTATCGTCC
Atf3	GAGCTGAGATTCGCCATCCA	CCGCCTCCTTTTCCTCTCAT
Ddit3	CCACCACACCTGAAAGCAGAA	AGGTGAAAGGCAGGGACTCA
Spliced Xbp1	CTGAGTCCGAATCAGGTGCAG	GTCCATGGGAAGATGTTCTGG
total Xbp1	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG
Rn18S	CATTAAATCAGTTATGGTTCCTTTGG	CCCGTCGGCATGTATTAGCT
<u>*Human*</u>		
TNFA	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
CCL2	CAGCCAGATGCAATCAATGCC	TGGAATCCTGAACCCACTTCT
CXCL1	CCAGCTCTTCCGCTCCTC	CACGGACGCTCCTGCTG

Supplemental Figure I (related to Figure 1)

A

rHDL effect on gene expression (fold change >1.5, FDR ≤ 0.05)



B

rHDL-induced genes

Motif	Known match	p-value	q-value
	MITF	1E-10	<0.0001
	Usf2	1E-9	<0.0001
	CLOCK	1E-9	<0.0001
	TFE3	1E-8	<0.0001
	bHLHE40	1E-8	<0.0001
	JunB	1E-2	0.0385
	CEBP	1E-2	0.0578
	AP-1	1E-2	0.0845

C

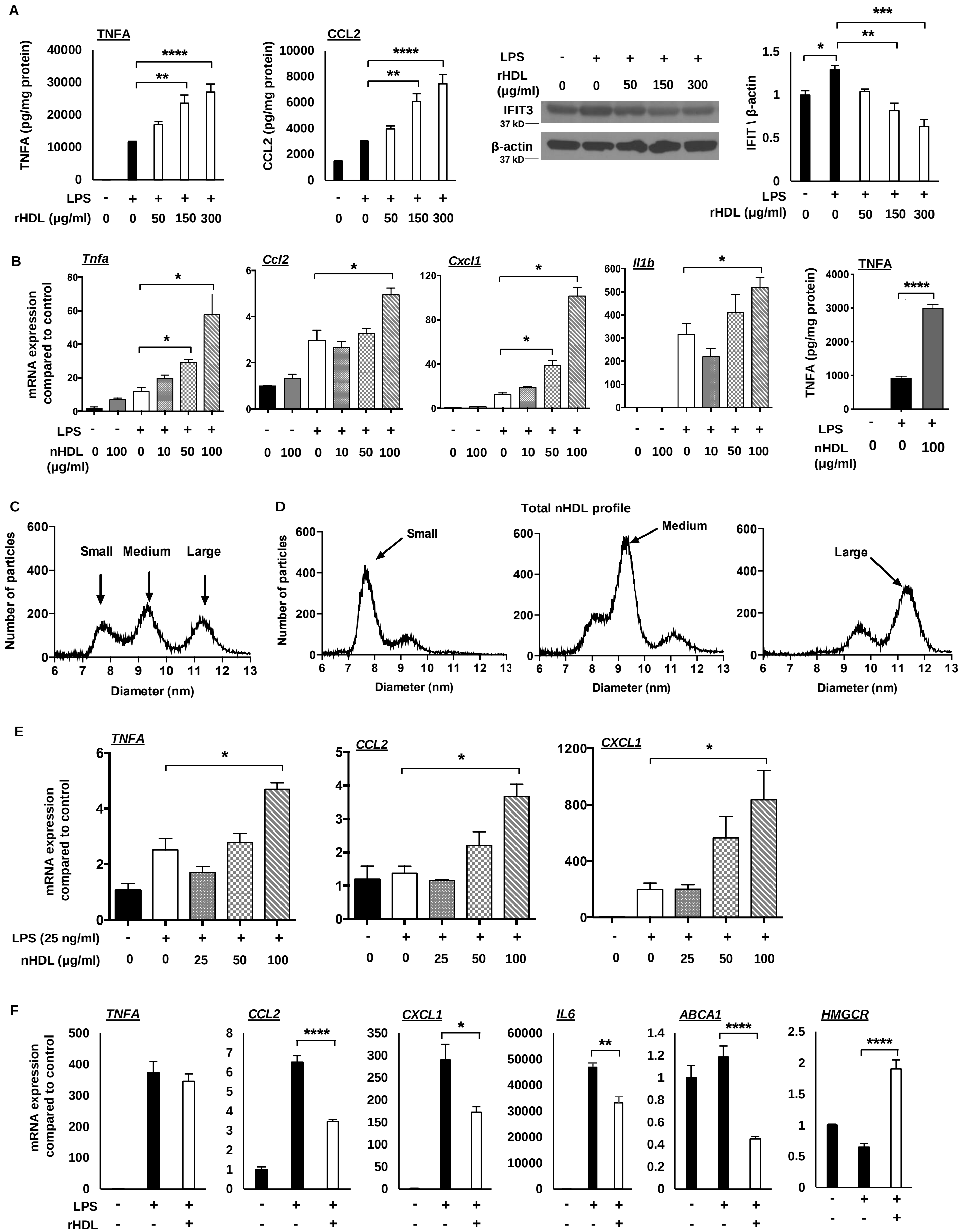
rHDL-repressed genes

Motif	Known match	p-value	q-value
	ISRE	1E-21	<0.0001
	IRF1	1E-18	<0.0001
	IRF3	1E-18	<0.0001
	IRF2	1E-16	<0.0001
	IRF8	1E-13	<0.0001
	AP-1	1E-4	0.0043

Supplemental Figure I; Related to Figure 1

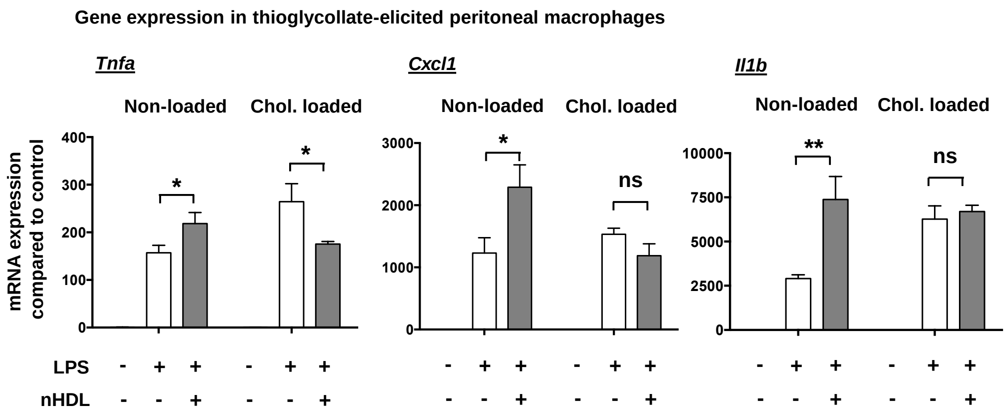
Wild type BMDMs were treated for 20 hours with 150 µg/ml rHDL (reconstituted HDL), washed with PBS and stimulated with 100 ng/mL LPS for 4 hours and harvested for RNA-Seq (n=3/condition). For cholesterol loading (CL) macrophages were incubated with POPC/cholesterol-liposomes (~ 1 mg cholesterol/ml) for 20 hours prior to rHDL treatment. (A) Overall gene expression changes from RNA-seq and (B, C) HOMER *de novo* motif enrichment in rHDL-induced genes and rHDL-repressed genes.

Supplemental Figure II (related to Figure 2)



Supplemental Figure II (continued) (related to Figure 2)

G



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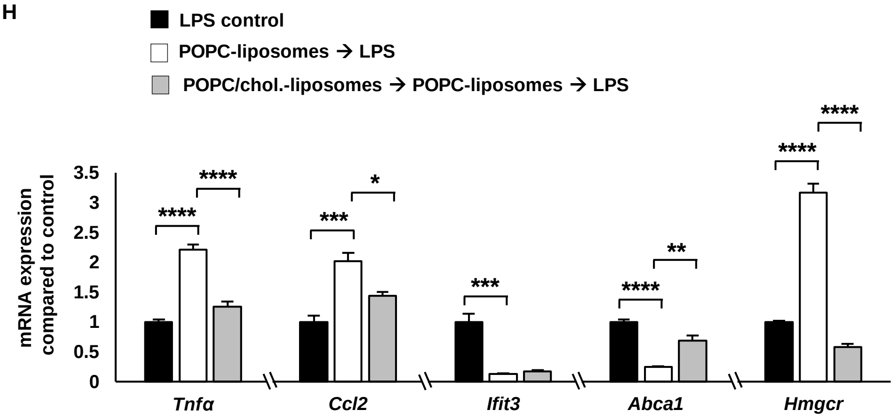
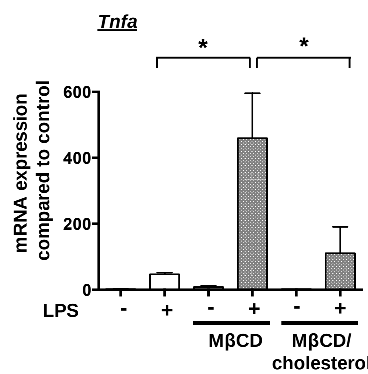


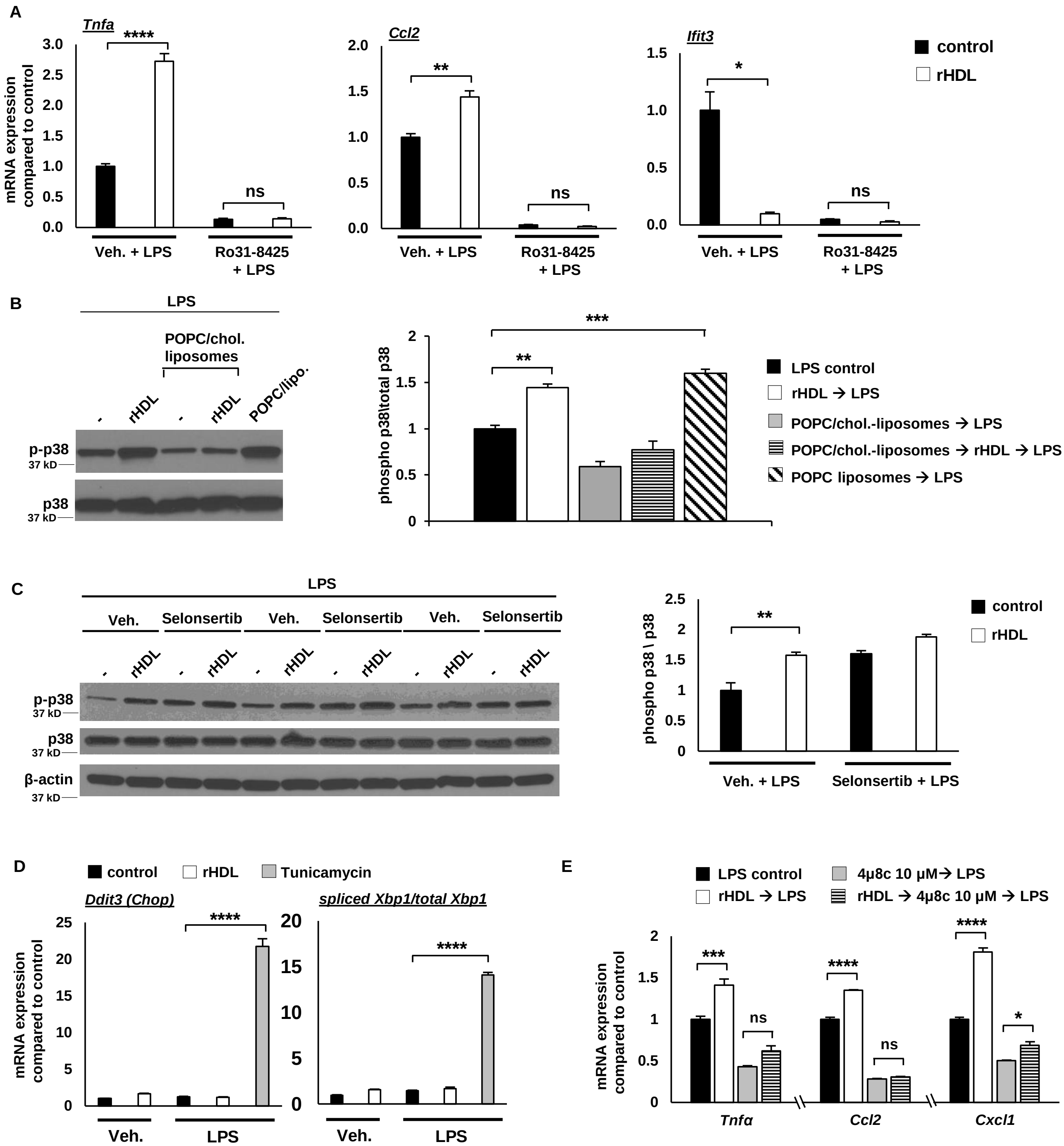
I



Supplemental Figure II; Related to Figure 2

Macrophages were treated for 18-20 hours with rHDL (reconstituted HDL) or nHDL (native HDL), washed with PBS and stimulated with LPS. For altering cholesterol content, macrophages were incubated with AcLDL prior to nHDL treatment. (A) Dose effect of rHDL on TNFA and CCL2 secretion and IFIT3 protein expression in LPS-stimulated (100 ng/ml, 4 hours) BMDMs, (B) Dose effect of isolated total nHDL on LPS-stimulated (10 ng/ml, 4 hours) inflammatory gene expression and TNFA secretion in BMDMs. (C) Profile of nHDL isolated from *APOA1^{Tg} Ldlr$^{-/-}$* mice by ultracentrifugation determined by calibrated ion mobility analysis. (D) Profile of different nHDL subpopulations (small, medium and large) fractionated by FPLC, as determined by calibrated ion mobility analysis. (E) nHDL dose response in LPS-stimulated (25 ng/ml, 4 hours) THP-1 cells. (F) Effect of rHDL (150 µg/ml) on inflammatory gene expression in LPS-stimulated (100 ng/ml, 4 hours) human PBMC-derived macrophages. (G) Effect of nHDL on LPS-induced (10 ng/ml, 4 hours) inflammatory gene expression in thioglycollate-elicited macrophages loaded with cholesterol by AcLDL prior to nHDL treatment (50 µg/ml, 48 hours). (H) Effect of POPC-liposomes on LPS-induced (100 ng/ml, 4 hours) inflammatory gene expression in BMDMs pre-treated with POPC/cholesterol-liposomes (~ 1 mg cholesterol/ml) for 20 hours, prior to POPC-liposomes treatment. (I) Effect of methyl-β-cyclodextrin (MβCD) (10 µM, 4 hours) or MβCD complexed with cholesterol (100 µg/ml, 4 hours) on LPS-induced (10 ng/ml, 4 hours) *Tnfa* expression in BMDMs. The results are shown as mean ± SEM (n=4). Tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) were performed for each of the data sets. Significance was determined by one-way ANOVA with Tukey's multiple comparisons test (A, Western blot data, and B, E, F, G, H, I) or nonparametric Kruskal-Wallis with Dunn's multiple comparisons test (A, ELISA data), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of at least two independent experiments.

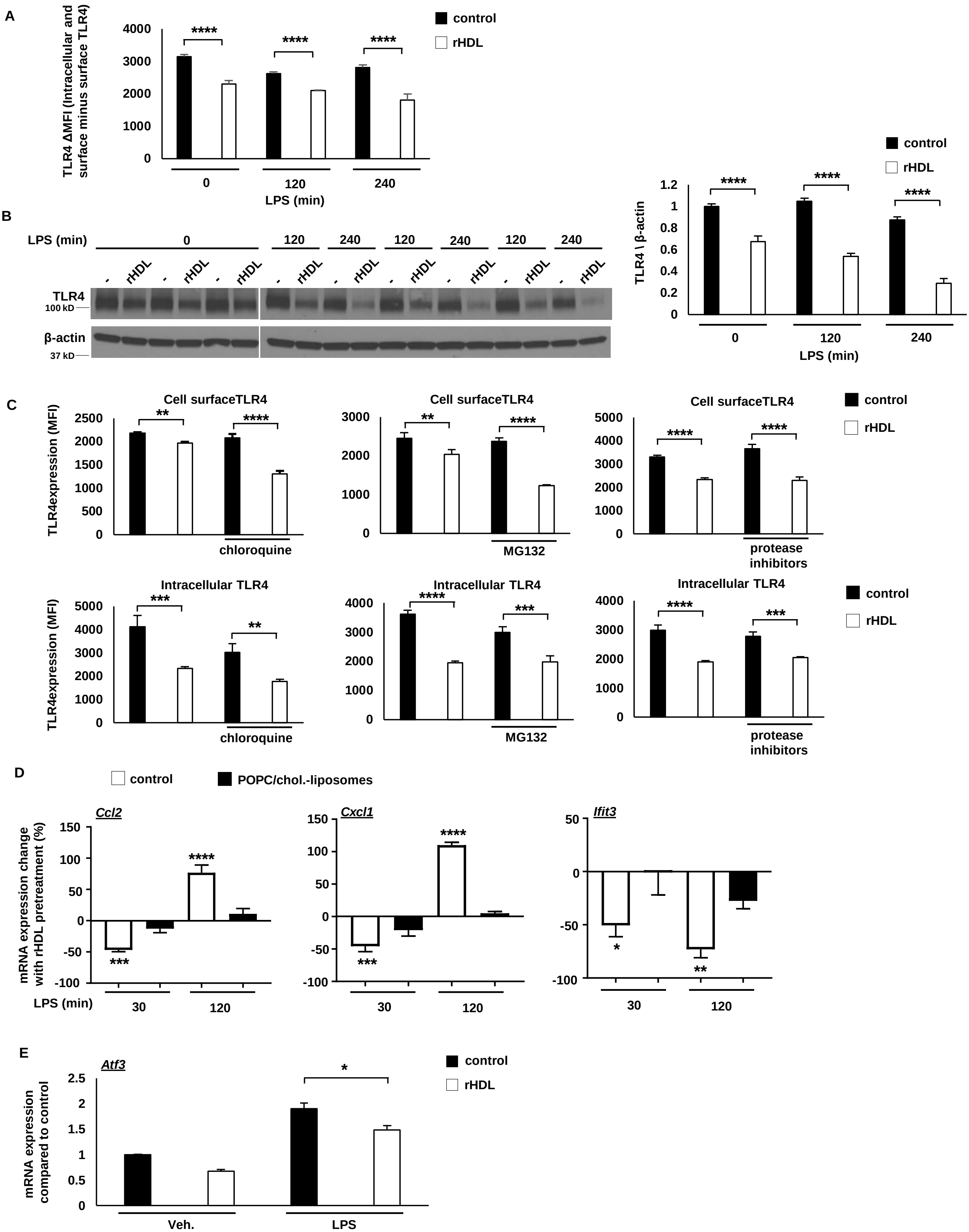
Supplemental Figure III (related to Figure 4)



Supplemental Figure III; Related to Figure 4

Wild type BMDMs were treated for 20 hours with rHDL, washed with PBS and stimulated with LPS, as described below. Macrophages were treated with the indicated inhibitors after removing rHDL and washing the cells, but prior to LPS stimulation and remained in the medium during the LPS stimulation. For altering cholesterol content, macrophages were incubated with POPC/cholesterol-liposomes (to load them with cholesterol) prior to rHDL treatment or cholesterol-free POPC-liposomes (instead of rHDL, to remove cholesterol). (A) Effect of rHDL (150 µg/ml) and Protein Kinase C (PKC) inhibitor (Ro31-8425, 10 µM, added 2 hours prior to LPS) on inflammatory gene expression in LPS-stimulated (100 ng/ml, 4 hours) BMDMs. (B) Effect of rHDL (150 µg/ml) on p38 MAPK phosphorylation in BMDMs pre-treated with POPC/cholesterol-liposomes (~ 1 mg cholesterol/ml) for 20 hours or cells pre-treated with POPC-liposomes alone and stimulated with LPS (100 ng/ml, 4 hours). (C) Effect of rHDL (150 µg/ml) and ASK1 inhibitor (Selonsertib, 10 µM, added 2 hours prior to LPS) on p38 MAPK phosphorylation in LPS-stimulated (100 ng/ml, 4 hours) BMDMs. (D) Effect of rHDL (300 µg/ml) and the ER stressor Tunicamycin (Tm, 2.5 µg/ml, added 2 hours prior to LPS) on ER stress gene expression in LPS-stimulated (100 ng/ml, 4 hours) BMDMs. (E) Effect of rHDL (300 µg/ml) and the IRE1a kinase inhibitor (4µ8c, 10 µM, added 1 hour prior to LPS) on inflammatory gene expression in LPS-stimulated (100 ng/ml, 4 hours) BMDMs. The results are shown as mean ± SEM (n=4 for gene expression, n=3 for protein expression). Tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) were performed for each of the data sets. Significance was determined by unpaired *t* test with Welch's correction (A), one-way ANOVA with Tukey's multiple comparisons test (B, C, D, E), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of at least two independent experiments.

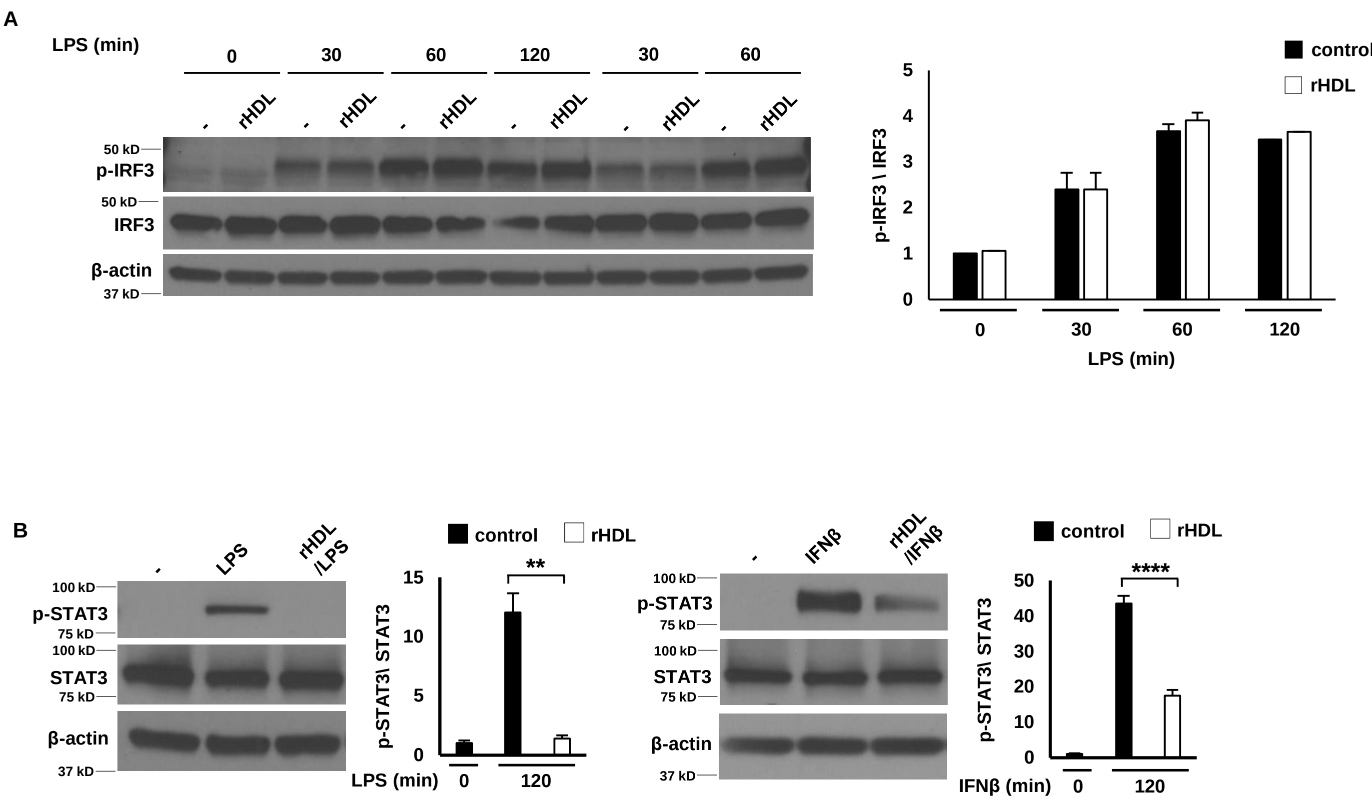
Supplemental Figure IV (related to Figure 5)



Supplemental Figure IV; Related to Figure 5

Wild type BMDMs were treated for 20 hours with rHDL, washed with PBS and stimulated with LPS, as described below. For altering cholesterol content, macrophages were incubated with POPC/cholesterol-liposomes prior to rHDL treatment to load them with cholesterol. (A) Effect of rHDL (150 µg/ml) on total TLR4 protein expression assessed by flow cytometry and (B) total TLR4 protein levels assessed by Western blot in LPS-stimulated (100 ng/ml) BMDMs at the indicated time points. (C) Effect of rHDL (150 µg/ml) on cell surface and intracellular TLR4 expression assessed by flow cytometry in BMDMs treated with chloroquine (50 µM) or MG132 (10 µM) or protease inhibitors (Pepstatin 10 µM, Leupeptin 20 µM, E64 20 µM, Calpepstin 10 µM). Chloroquine, MG132 or the protease inhibitors where added during the last 4-6 hours of the rHDL treatment. (D) Effect of rHDL (150 µg/ml) on inflammatory gene expression in LPS-stimulated (100 ng/ml, time points 30 and 120 minutes) in BMDMs pre-treated with POPC/cholesterol-liposomes (~ 1 mg cholesterol/ml) for 20 hours BMDMs prior to rHDL. Results are expressed as the change of mRNA expression in rHDL treated versus non-treated cells. (E) Effect of rHDL (150 µg/ml) on *Atf3* expression in LPS-stimulated (100 ng/ml, 4 hours) BMDMs. The results are shown as mean ± SEM (n=4 for gene expression, n=3 for protein expression). Tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) were performed for each of the data sets. Significance was determined by two-way ANOVA with Sidak's post-hoc test (A, B, D) or by one-way ANOVA with Tukey's multiple comparisons test (C, E), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are representative of at least two independent experiments.

Supplemental Figure V (related to Figure 5)

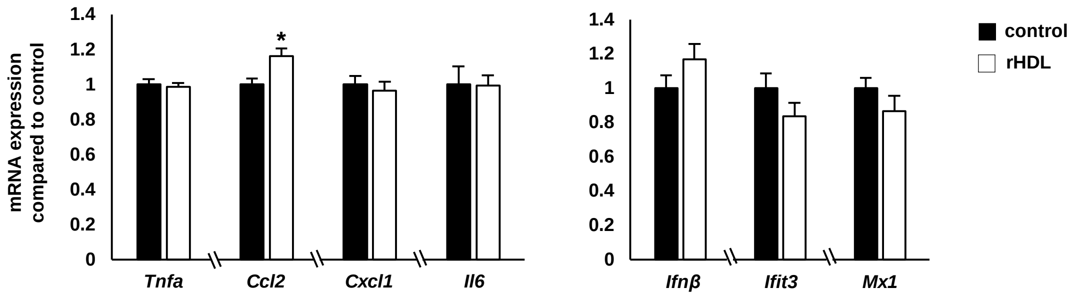


Supplemental Figure V; Related to Figure 5

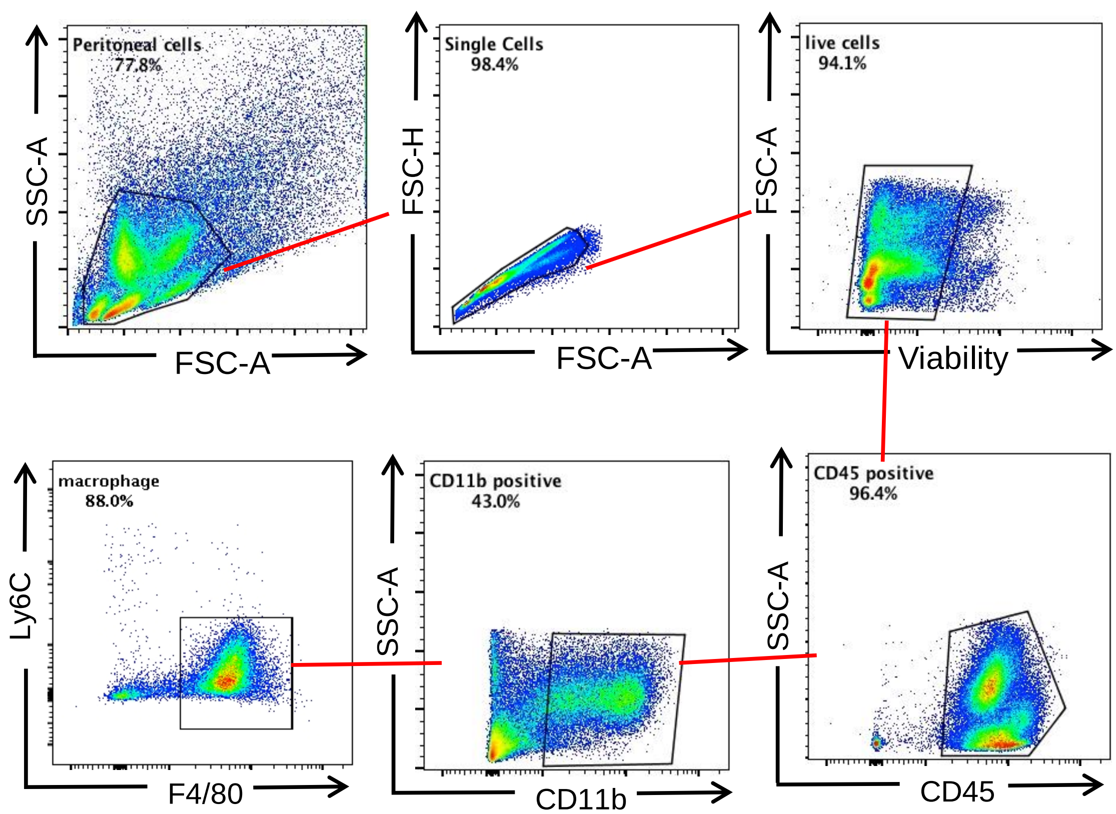
Wild type BMDMs were treated for 20 hours with rHDL, washed with PBS and stimulated with LPS, as described below. (A) Effect of rHDL (150 µg/ml) on IRF3 phosphorylation in LPS-stimulated (100 ng/ml) BMDMs at the indicated time points. (B) Effect of rHDL (150 µg/ml) on STAT3 phosphorylation in LPS-stimulated (100 ng/ml) or IFNb-stimulated (5 ng/ml, 2 hours) BMDMs. The results are shown as mean ± SEM (n=3). Tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) were performed for each of the data sets. Significance was determined by two-way ANOVA with Sidak's post-hoc test (A) or by unpaired *t* test with Welch's correction (B), $^{**}p<0.01$, $^{****}p<0.0001$. Data are representative of at least two independent experiments.

Supplemental Figure VI (related to Figure 6)

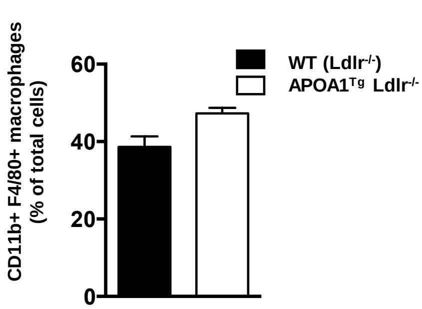
A Thioglycollate elicited peritoneal macrophages from Ldlr-/- mice fed WTD diet for 8 weeks stimulated with LPS



B Gating scheme for resident peritoneal cells from WT (Ldlr-/-) and APOA1^{TG} Ldlr-/- mice



Peritoneal macrophages in WT and APOA1^{TG} mice before adhesion purification



Supplemental Figure VI; Related to Figure 6

(A) *Ldlr*$^{-/-}$ mice fed a WTD diet for 8 weeks were injected intravenously with 80 mg/Kg rHDL (n=5) or PBS daily (n=5), for a total of 5 days before sacrificing the mice 2 hours after the last rHDL injection. Thioglycollate was injected intraperitoneally 3 days before sacrificing the mice and peritoneal macrophages were collected, stimulated with LPS (100 ng/ml, 2 h) and inflammatory gene expression was assessed. (B) CD11b+ F4/80+ resident macrophages from *Ldlr*$^{-/-}$ and *APOA1*^{Tg}*;Ldlr*$^{-/-}$*;* mice (n=4). The gating scheme for characterization of resident peritoneal macrophages is shown, as is the % macrophages (Cd11b+ F4/80+ population) before adhesion purification. The results are shown as mean ± SEM. Significance was determined by multiple t-tests using the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 5%, *p<0.05

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J	Female
B6.129P2-Apoetm1Unc/J	The Jackson Laboratory Cat# 002052	C57BL/6J	Female
B6.129S7-Ldlrtm1Her/J	The Jackson Laboratory Cat# 002207	C57BL/6J	Female
B6.129S2-Ifnar1tm1Agt/Mmjax	The Jackson Laboratory Cat# 32045-JAX	C57BL/6J	Female
C57BL/6-Tg(APOA1)1Rub/J	The Jackson Laboratory Cat# 001927	C57BL/6J	Female

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J	
Parent - Female	C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J	
Parent - Male	B6.129S7-Ldlrtm1Her/J	The Jackson Laboratory Cat# 002052	C57BL/6J	These mice we crossed to generate APOA1Tg Ldlr-/- and Ldlr-/- littermates
Parent - Female	C57BL/6-Tg(APOA1)1Rub/J	The Jackson Laboratory Cat# 001927	C57BL/6J	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
IκBα	Cell signaling	Cat # 9242	Not available 1:1000 from stock	
p-p38 MAPK	Cell signaling	Cat # 4511	Not available 1:1000 from stock	
p38 MAPK	Cell signaling	Cat # 8690	Not available 1:2000 from stock	
p-ERK1/2	Cell signaling	Cat # 4370	Not available 1:1000 from stock	
ERK1/2	Cell signaling	Cat # 4695	Not available 1:2000 from stock	
p-JNK1/2	Cell signaling	Cat # 4668	Not available 1:1000 from stock	

JNK1/2	Cell signaling	Cat # 9252	Not available 1:1000 from stock	
p-IRF3	Cell signaling	Cat # 4947	Not available 1:1000 from stock	
IRF3	Cell signaling	Cat # 4302	Not available 1:2000 from stock	
p-IRF7	Cell signaling	Cat # 24129	Not available 1:500 from stock	
IRF7	Abcam	Cat # ab62505	Not available 1:1000 from stock	
p-STAT1	Cell signaling	Cat # 9167	Not available 1:1000 from stock	
STAT1	Cell signaling	Cat # 14994	Not available 1:2000 from stock	
p-STAT3	Cell signaling	Cat # 9131	Not available 1:1000 from stock	
STAT3	Cell signaling	Cat # 4904	Not available 1:2000 from stock	
Ire1a	Cell signaling	Cat # 3294	Not available 1:500 from stock	
TLR4	Cell signaling	Cat # 14358	Not available 1:1000 from stock	
IFNAR1	Santa Cruz Biotechnology	Cat # sc-7391	0.4 µg/ml	
IFIT3	EMD Millipore	Cat # 1 ABF1048	Not available 1:1000 from stock	
β-actin	Sigma	Cat # A5441	Not available 1:8000 from stock	
anti-rabbit IgG HRP-linked antibody	Cell signaling	Cat # 7074	Not available 1:5000 from stock	
anti-mouse IgG HRP-linked antibody	GE Healthcare	Cat # NA931	Not available 1:10000 from stock	
F4/80-Pacific Blue clone BM8	Biolegend	Cat # 123124	2.5 µg/ml	
TLR4-PE/Cy7 clone SA15-21	Biolegend	Cat # 145408	1 µg/ml	
anti–CD16/CD32 mAb	eBiosiences	Cat # 14-0161-82	0.25 µg/ml	
PE-Cy7-labeled F4/80 clone BM8	eBiosiences	Cat # 25-4801-82	0.1 µg/ml	
APC-labeled Ly-6C clone 1A8	eBiosiences	Cat # 17-5932-80	0.1 µg/ml	
PE-labeled CD11b clone M1/70	eBiosiences	Cat # 12-0112-82	0.05 µg/ml	
FITC-labeled CD45 clone 30-F11	eBiosiences	Cat # 11-0451-82	0.25 µg/ml	

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Human THP-1 monocytic cell line	ATCC Cat# ATCC® TIB-202	Male
mouse J774 macrophages	ATCC Cat# J774A.1 (ATCC® TIB-67)	Female
Bone marrow derived macrophages	Derived from mouse strains described above	Female
Peritoneal macrophages	Derived from mouse strains described above	Female

Other reagents and commercial assays

Name	Vendor or Source	Catalog #
Lipopolysaccharide	Cell Signaling	Cat# 14011
Lipopolysaccharide ultrapure	List Biological Laboratories	Cat# NC9633766
phorbol 12-myristate 13-acetate (PMA)	Sigma	Cat# P8139
Lipofectamine RNAiMAX	Thermo	Cat# 13778075
BIRB0796	AXON Medchem	Cat# 1358
Recombinant mouse IFNβ	R&D systems	Cat# 8234-MB
Thioglycollate	BD	Cat# BD 292788
Selonsertib	Selleckchem	Cat# S8292
Sodium phenylbutyrate (4PBA)	Sigma	Cat# SML0309
IRE1 Inhibitor IV, KIRA6	EMD Millipore	Cat# 532281
IRE1 Inhibitor III, 4μ8C	EMD Millipore	Cat# 412512
PKC inhibitor Ro31-8425	Sigma	Cat# 557514
Tunicamycin	Sigma	Cat# SML1287
Chloroquine	Invivogen	Cat# tlrl-chq
MG132	Sigma	Cat# M7449
Pepstatin	Sigma	Cat# P5318
Leupeptin	Sigma	Cat# L5793
E64	Sigma	Cat# E3132
Calpeptin	Sigma	Cat# C8999
Halt Protease inhibitor cocktail	Thermo	Cat# 1861278
Halt phosphatase inhibitor cocktail	Thermo	Cat# 78427
methyl-β-cyclodextrin	Sigma	Cat# C4555
methyl-β-cyclodextrin complexed with cholesterol	Sigma	Cat# C4951
Fast SYBR Green Master Mix	Thermo	Cat# 4385612
Cholesterol	Sigma	Cat# C3045
1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)	Avanti Polar lipids	Cat# 850457
human acetylated LDL	Kalen Biomedical	Cat# 770201
cyclic AMP	Sigma	Cat# A6885
acyl-coenzyme A:cholesterol acyltransferase inhibitor (Sandoz 58-035)	Sigma	Cat# S9318
BODIPY 493/503	Invitrogen	Cat# D3922
Liberase TH	Roche	Cat# 5401151001
Hyaluronidase	Sigma	Cat# H3506

Deoxyribonuclease I from bovie	Sigma	Cat# DN25
BD Cytofix/Cytoperm	BD Biosciences	Cat# 554722
BD Perm/Wash	BD Biosciences	Cat# 554723
RNeasy mini kit	Qiagen	Cat # 74106
recombinant human M-CSF	Peprotech	Cat # 300-25
Human Serum	Sigma	Cat # H4522
Ficoll-Pague Plus	GE Healthcare	Cat # 17144002
Quick-RNA Miniprep	Zymo Research	Cat# R1055
Nucleospin RNA plus	Takara Bio	Cat# 740984
NEBNext RNA Ultra library prep kit	NEB	Cat# E7530S
BCA protein assay kit	Pierce	Cat# 23225
Supersignal West Pico Chemiluminescent substrate	Pierce	Cat# 34578
CD11b microbeads, mouse (and human)	Miltenyi Biotec	Cat# 130-049-601
Cholesterol E (total cholesterol assay)	Wako Diagnostics	Cat# 999-02601
Maxima First Strand cDNA synthesis kit for RT-qPCR	Thermo	Cat# K1642
Mouse TNF-alpha DuoSet ELISA	R&D	DY410-05
Mouse CCL2/JE/MCP-1 DuoSet ELISA	R&D	DY479-05

Oligonucleotides

Name	Vendor or Source	Catalog #
Primers for Quantitative PCR , see Supplemental Table V	This paper	
Antisense LNA Gapmer oligonucleotide targeting mouse Ask1 (NM_008580.4): GATAGATTTTGGTTGG	Qiagen	339511 LG00214119-DDA
Antisense LNA Gapmer oligonucleotide negative control A: AACACGTCTATACGC	Qiagen	339515 LG00000002-DDA

Software and algorithms

Name	Vendor or Source	link
Graphpad Prism v7.0.3	GraphPad Software	https://www.graphpad.com/scientificsoftware/prism/
PANTHER GO	Thomas et al., 2003	http://www.geneontology.org/page/goenrichment-analysis
HOMER v4.9.1	Heinz et al., 2010	http://homer.ucsd.edu/homer/

Deposited data

Name	Vendor or Source	link
rHDL RNA-seq	This paper	GEO: GSE129347