Statistics

Unless otherwise indicated data are presented as mean ± standard error of mean (s.e.m.). For statistical analysis, two-tailed, unpaired Student's t-test and ANOVA for multiple comparisons were employed. Post-hoc comparisons were performed with the Tukey test. $p<0.05$ was considered statistically significant. All significant p-values are included in Table S4. Basic analyses were performed with Excel (Microsoft) and Prism (GraphPad Software, Inc.). Furthermore, Volocity Quantitation (PerkinElmer) was used for image analysis and gene expression data was analyzed with Partek Genomics Suite (Partek) using ANOVA models and GSEA results were plotted using Sigmaplot 12.2.

Supplementary Materials:

Fig. S1. CD treatment does not influence general cardiovascular parameters.

Fig. S2. CD does not alter plasma cholesterol metabolite and phytosterol concentrations in atherosclerosis regression and treatment trials.

Fig. S3. 10 mM CD does not affect viability of murine macrophages.

Fig. S4. CD mediates intracellular CC dissolution.

Fig. S5. CC loading of macrophages induces lipid droplet accumulation.

Fig. S6. CD treatment induces the expression of cholesterol efflux transporters in the aortic arch of atherosclerotic mice.

Fig. S7. CD treatment does not alter murine lipoprotein profiles.

Table S1. LXR target gene list for GSEA analysis of BMDMs from WT and LXR$\alpha^{-/-}\beta^{-/-}$ mice.

Table S2. List of additional metabolic and regulatory genes (nCounter Panel Plus).

Table S3. LXR target gene list for GSEA analysis of human atherosclerotic plaques.

Table S4. Original data of all figures.

References:

1. J. G. Robinson, M. Farnier, M. Krempf, J. Bergeron, G. Luc, M. Averna, E. S. Stroes, G. Langslet, F. J. Raal, M. E. Shahawy, M. J. Koren, N. E. Lepor, C. Lorenzato, R. Pordy, U. Chaudhari, J. J. P. Kastelein, ODYSSEY LONG TERM Investigators, Efficacy and Safety of Alirocumab in Reducing Lipids and Cardiovascular Events, *N Engl J Med* (2015), doi:10.1056/NEJMoa1501031.

2. M. S. Sabatine, R. P. Giugliano, S. D. Wiviott, F. J. Raal, D. J. Blom, J. Robinson, C. M. Ballantyne, R. Somaratne, J. Legg, S. M. Wasserman, R. Scott, M. J. Koren, E. A. Stein, Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators, Efficacy and Safety of Evolocumab in Reducing Lipids and Cardiovascular Events, *N Engl J Med*, 150315080057008 (2015).

Supplementary Materials:

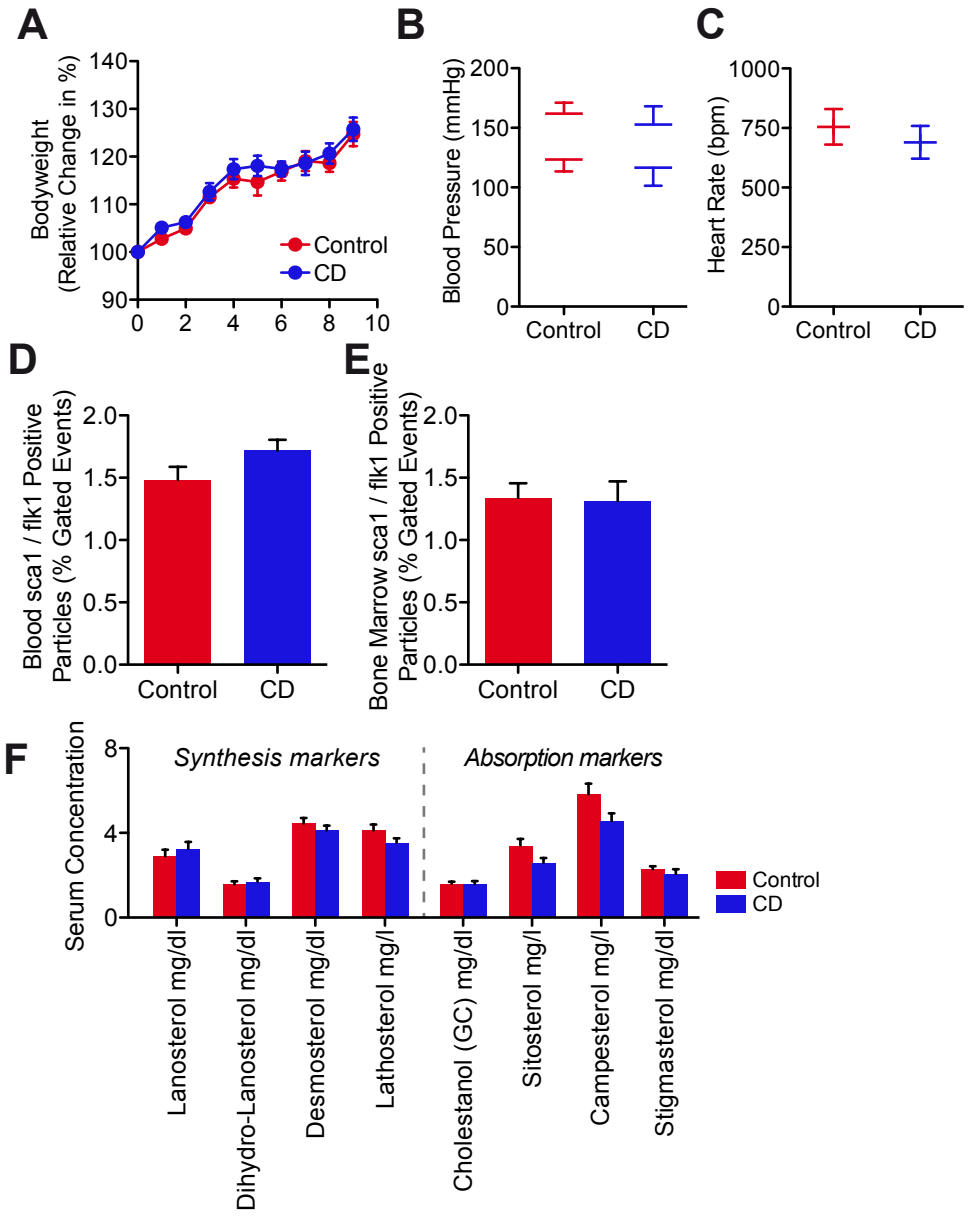

A
Bodyweight (Relative Change in %)
140
130
120
110
100
90
0
2
4
6
8
10
Control
CD
B
Blood Pressure (mmHg)
200
150
100
50
0
Control
CD
C
Heart Rate (bpm)
1000
750
500
250
0
Control
CD
D
Blood sca1 / flk1 Positive Particles (% Gated Events)
2.0
1.5
1.0
0.5
0.0
Control
CD
E
Bone Marrow sca1 / flk1 Positive Particles (% Gated Events)
2.0
1.5
1.0
0.5
0.0
Control
CD
F
Serum Concentration
8
4
0
Synthesis markers
Absorption markers
Lanosterol mg/dl
Dihydro-Lanosterol mg/dl
Desmosterol mg/dl
Lathosterol mg/l
Cholestanol (GC) mg/dl
Sitosterol mg/l
Campesterol mg/l
Stigmasterol mg/dl
Control
CD

Fig. S1

Fig. S1. CD treatment does not influence general cardiovascular parameters. ApoE$^{-/-}$ mice were fed a cholesterol-rich diet for eight weeks and concomitantly treated with 2 g CD/ kg body weight or vehicle control twice a week (n=7-8 per group). **(A)** Weekly change in bodyweight relative to starting weight. **(B)** Blood pressure and **(C)** heart rate in the final week of CD or control treatment. Percent of **(D)** circulating and **(E)** bone marrow sca1/flk1 positive cells relative to the number of gated events. **(F)** Plasma concentration of cholesterol precursors as "synthesis markers", the metabolite cholestanol and selected phytosterols as "absorption markers". Data are shown as mean + s.e.m. or mean +/- s.e.m.

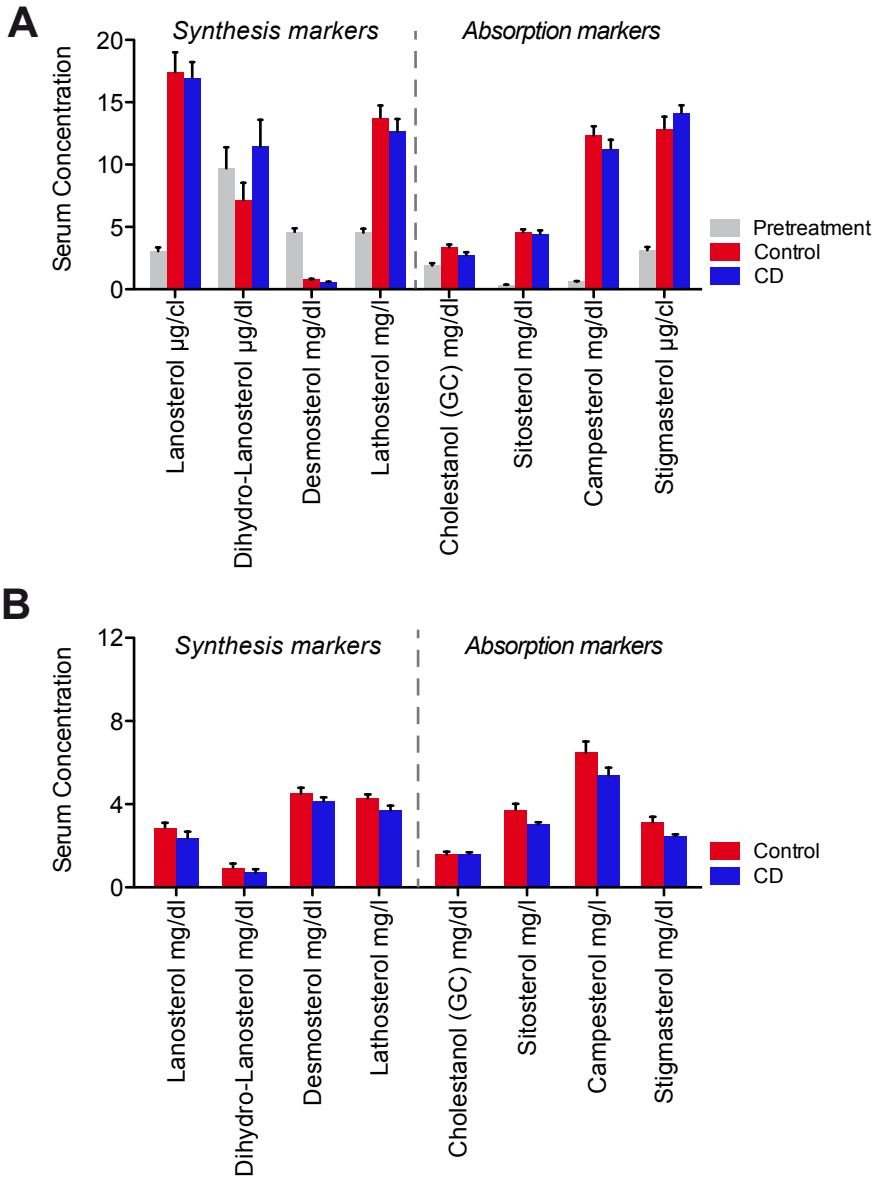

A
Synthesis markers
Absorption markers
Serum Concentration
20
15
10
5
0
Pretreatment
Control
CD
Lanosterol µg/dl
Dihydro-Lanosterol µg/dl
Desmosterol mg/dl
Lathosterol mg/l
Cholestanol (GC) mg/dl
Sitosterol mg/dl
Campesterol mg/dl
Stigmasterol µg/dl
B
Synthesis markers
Absorption markers
Serum Concentration
12
8
4
0
Control
CD
Lanosterol mg/dl
Dihydro-Lanosterol mg/dl
Desmosterol mg/dl
Lathosterol mg/l
Cholestanol (GC) mg/dl
Sitosterol mg/l
Campesterol mg/l
Stigmasterol mg/dl

Fig. S2

Fig. S2. CD treatment does not alter plasma sterol concentrations in atherosclerosis regression trials. Plasma concentration of cholesterol precursors as "synthesis markers", the metabolite cholestanol and selected phytosterols as "absorption markers" in **(A)** regression and **(B)** treatment trials (n=6-8 per group). Data are shown as mean + s.e.m.

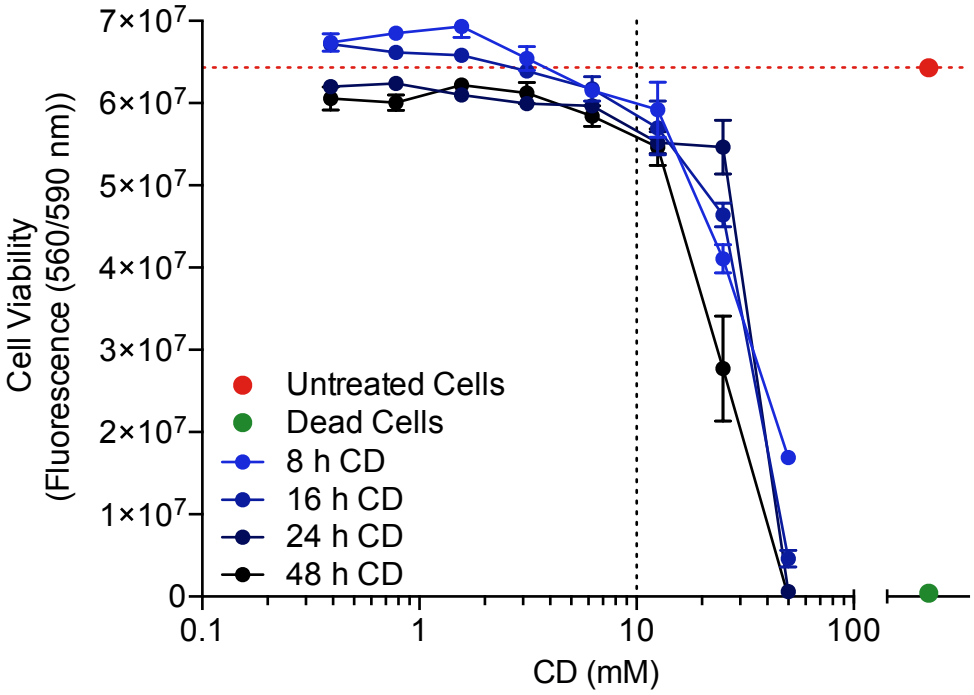

Cell Viability
(Fluorescence (560/590 nm))
7×10^7
6×10^7
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
0.1
1
10
100
CD (mM)
Untreated Cells
Dead Cells
8 h CD
16 h CD
24 h CD
48 h CD

Fig. S3

Fig. S3. 10 mM CD does not affect the viability of murine macrophages. Cell viability of iMacs incubated for indicated times with increasing concentrations of CD ranging from 0.391 mM to 50 mM. Dashed line indicates commonly used CD concentration (10 mM). Data are shown as mean +/- s.e.m.

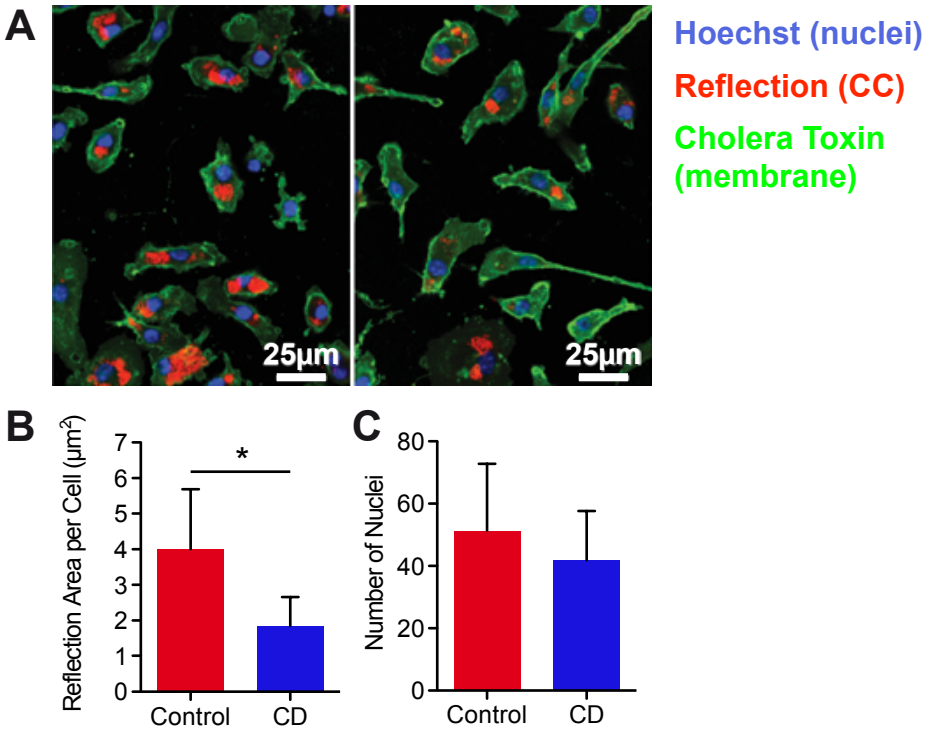

A
25µm
25µm
Hoechst (nuclei)
Reflection (CC)
Cholera Toxin (membrane)
B
Reflection Area per Cell (µm²)
0
1
2
3
4
5
6
7
*
Control
CD
C
Number of Nuclei
0
20
40
60
80
Control
CD

Fig. S4

Fig. S4. CD mediates intracellular CC dissolution. BMDMs were loaded with 100 μg CC per $1x10^6$ cells for 3 h and incubated with control or CD for 24 h. Nuclei were stained with Hoechst (blue) and the plasma membrane was visualized using Alexa Fluor 647–coupled cholera toxin subunit B (green). The cells were imaged using confocal laser reflection microscopy. Intracellular CC reflection signal (red) was determined by automated image quantification based on cell mask. Data are shown as mean + s.e.m, Control vs. CD, unpaired one-tailed Student's t test; *$p < 0.05$.

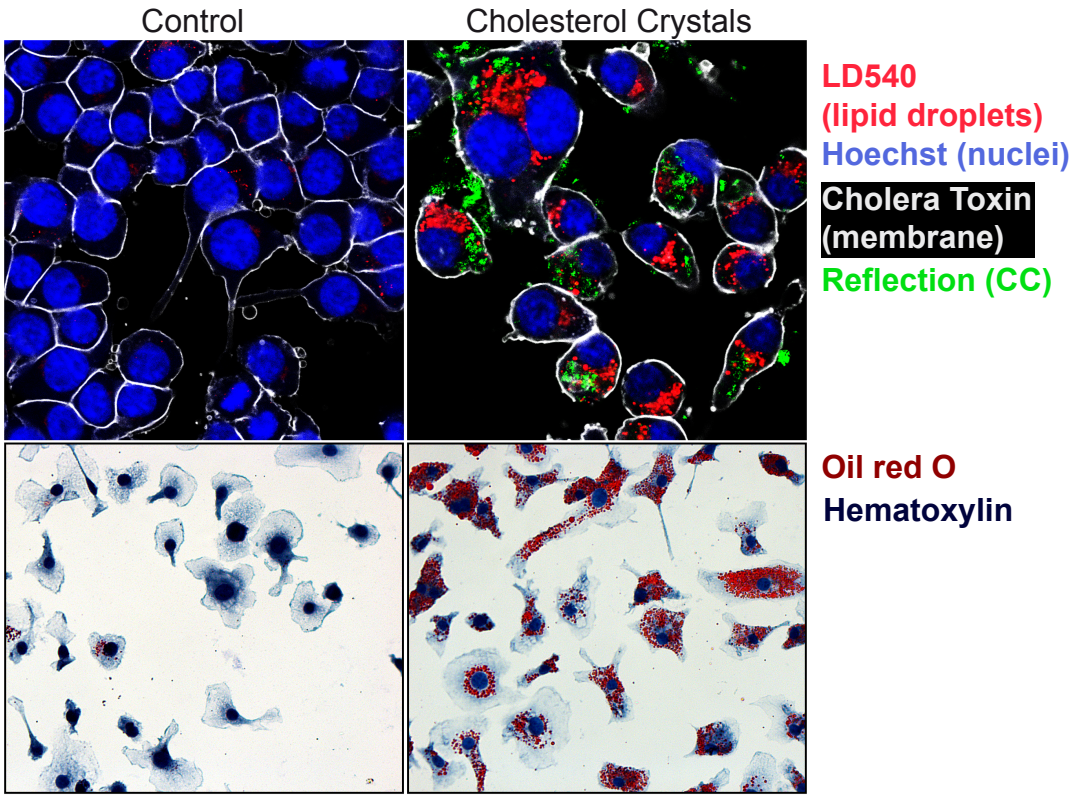

Control
Cholesterol Crystals
LD540
(lipid droplets)
Hoechst (nuclei)
Cholera Toxin
(membrane)
Reflection (CC)
Oil red O
Hematoxylin

Fig. S5

Fig. S5. CC loading of macrophages induces lipid droplet accumulation. iMacs were loaded with 20 μg CC for 3 h. To visualize lipid droplet formation, resting and CC-loaded macrophages were imaged following two different staining techniques. Top: Macrophages were stained for lipid droplets with LD540 *(46)* (red), for nuclei with Hoechst (blue) and the plasma membrane was stained with Alexa Fluor 647–coupled cholera toxin subunit B (white). The cells were imaged using confocal laser reflection microscopy; reflection signal of CCs (green). Bottom: Macrophages were stained for neutral triglycerides and lipids with Oil Red O (red), counterstained with Hematoxylin (dark blue).

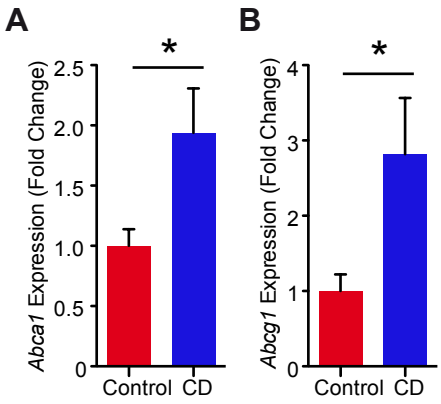

A
B
*
*
Abca1 Expression (Fold Change)
Abcg1 Expression (Fold Change)
2.5
2.0
1.5
1.0
0.5
0
4
3
2
1
0
Control
CD
Control
CD

Fig. S6

Fig. S6. CD treatment induces the expression of cholesterol efflux transporters in aortic arches of atherosclerotic mice. Gene expression of **(A)** *Abca1* and **(B)** *Abcg1* in aortic tissue of $ApoE^{-/-}$ mice after eight weeks of cholesterol-rich diet and concomitantly treated with 2 g CD/ kg body weight or vehicle control twice a week (n=7-8 per group). Data are shown as mean + s.e.m., Control vs. CD, unpaired two-tailed Student's t test; *$p < 0.05$.

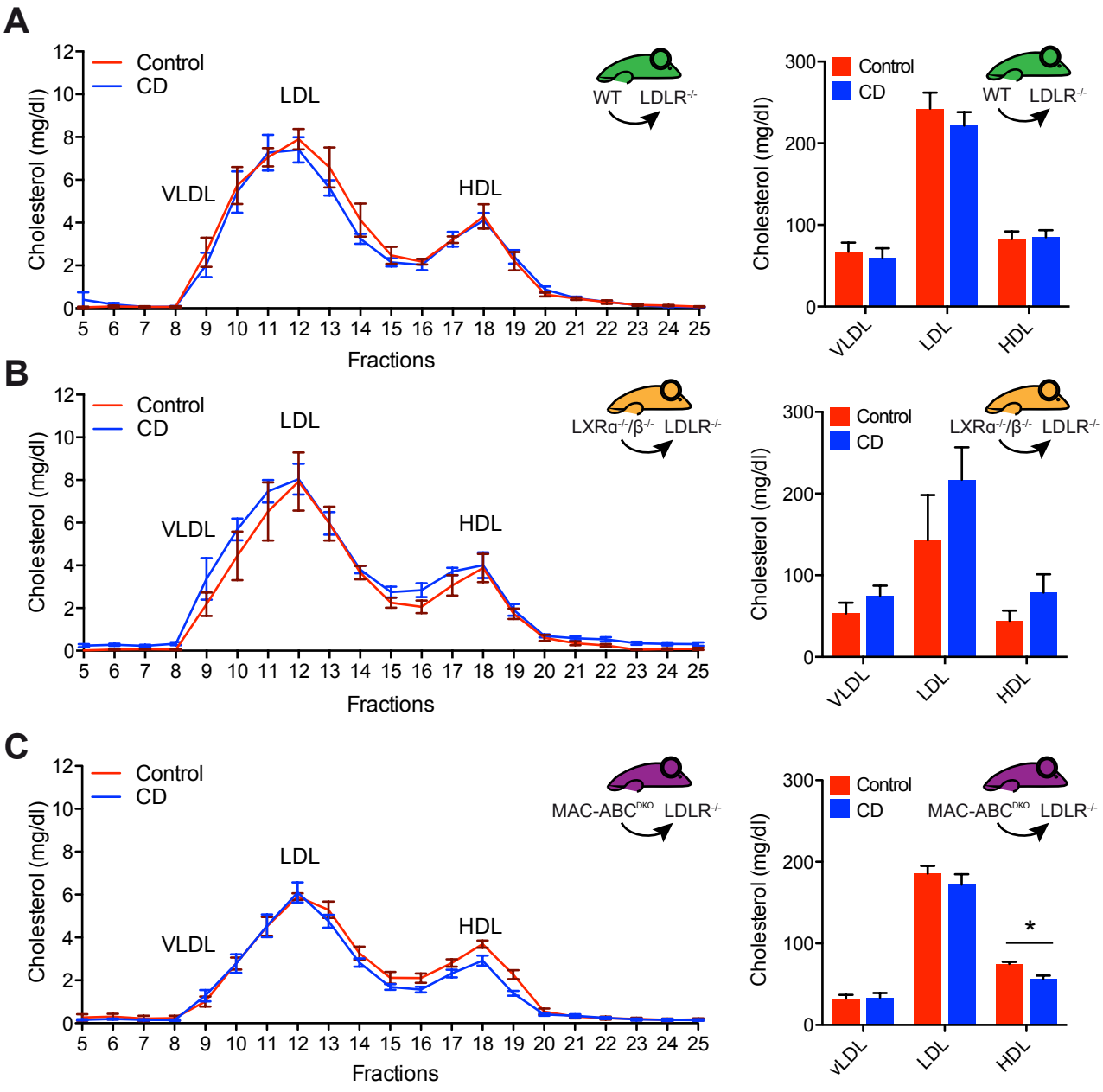

A
Control
CD
LDL
VLDL
HDL
Cholesterol (mg/dl)
Fractions
WT LDLR-/-
B
LXRα-/-/β-/- LDLR-/-
C
MAC-ABCDKO LDLR-/-
*
VLDL
LDL
HDL

Fig. S7

Fig. S7. CD treatment does not alter murine lipoprotein profiles. Lipoprotein profiles of $LDLR^{-/-}$ mice transplanted with **(A)** WT, **(B)** $LXR\alpha^{-/-}\beta^{-/-}$ or **(C)** $MAC\text{-}ABC^{DKO}$ bone marrow and fed a cholesterol-rich diet for eight weeks concomitantly treated with 2 g CD/ kg body weight or vehicle control twice a week (n=6-8 per group). Data are shown as mean + s.e.m. or mean +/- s.e.m.

Table S1. LXR target gene list for GSEA analysis of BMDMs from WT and $LXR\alpha^{-/-}\beta^{-/-}$ mice. The list contains 533 unique LXR target genes identified in Heinz *et al. (30)* covered by the Illumina Array.

LXR target gene names	Accession no.
Abcg1	NM_009593
2310035K24Rik	NM_027129.2
Col4a3bp	NM_023420.1
2310005P05Rik	NM_026189.2
Ptpro	NM_011216.2
Rgl1	NM_016846.3
Psap	NM_011179.2
5033414K04Rik	NM_001003948.1
Scd1	NM_009127.3
Cirbp	NM_007705.2
Stx8	NM_018768.2
Osgin1	NM_027950.1
Acsl3	NM_028817.2
1110032E23Rik	NM_133187.2
Klf9	NM_010638.4
2810439F02Rik	AK080904
Fbxo32	NM_026346.1
Acly	NM_134037.2
Chd9	NM_177224.1
Tmem86a	NM_026436.3
Sgk1	NM_011361.1
Ermp1	NM_001081213.1
Slc15a3	NM_023044.1
Cd28	NM_007642.2
Cpeb2	NM_175937.2
Irf8	NM_008320.3
Fut8	NM_016893.4
Scotin	NM_025858.1
Slc1a4	NM_018861.2
Sesn1	NM_001013370.1
D6Wsu176e	NM_138587.4
Sag	NM_009118.2
Acp2	NM_007387.1

Tlr4	NM_021297.1
Tmem120a	NM_172541.2
Endod1	NM_028013.2
Ccnd3	NM_007632.2
Tgfbr2	AK090393
Nnat	AK077465
Bcar3	NM_013867.1
Fgd2	NM_013710.3
Cd63	NM_007653.1
2610307O08Rik	XM_921606.2
Lrrk1	NM_146191.3
Snx27	NM_029721.1
Aebp2	AK045838
Traf3ip2	NM_134000.3
Abca1	NM_013454.3
Pdgfb	NM_011057.2
Chd2	NM_001081345.1
Tatdn2	NM_001033463.1
Lpin1	NM_015763
1700025G04Rik	NM_197990.2
Atp1a1	NM_144900.1
Tnfaip2	NM_009396.1
6430548M08Rik	NM_172286
Dusp6	NM_026268.2
Pik3ap1	NM_031376.2
Aldh4a1	NM_175438.3
Gpx1	NM_008160.5
3830408P04Rik	NM_023647
Ripk5	NM_172516.4
Rheb	NM_053075.2
Fam129b	NM_146119.1
Mafk	NM_010757.2
2810046L04Rik	NM_173382
Mylc2b	NM_023402.1
Ptgs1	NM_008969.3
Txnip	NM_023719.1
Hif1a	NM_010431.1
Tspan14	NM_145928.1
2010107E04Rik	NM_027360.2
Ly86	NM_010745.1
Irf2	NM_008391.3
5430435G22Rik	NM_145509.2
A530088I07Rik	AK080244
Agpat4	NM_026644.1
Tmc6	NM_145439.1
Cyth4	NM_028195.3
4921513D23Rik	NM_001081154

Atp2a2	NM_009722.2
Nob1	NM_026277.1
Ccdc109b	NM_025779.2
9430041J06Rik	NM_001081045
Prkcd	NM_011103.2
Insr	AK052187
Bex6	NM_001033539.2
Slc15a4	NM_133895.1
Ptprs	NM_011218.1
Tmem17	NM_153596.1
Vac14	NM_146216.2
Prkar1a	NM_021880.2
Uck2	NM_030724.3
Sh3yl1	NM_013709.4
Erlin2	NM_153592.1
Cpsf6	NM_001013391.1
Bola3	NM_175277.2
Sdc1	NM_011519.2
Nrbf2	NM_001036293.2
N6amt1	NM_026366.1
Mylip	NM_153789.3
Bcat1	NM_007532.3
Aoah	NM_012054.2
Srebf1	NM_011480.1
Cdkn2aipnl	NM_029976.2
Ak2	NM_016895.3
Mdm1	NM_148922.2
Adcy7	NM_007406.1
Ikbke	NM_019777.3
Tnfaip3	NM_009397.2
Gng10	NM_025277.3
Adrbk1	NM_130863.1
Scd2	NM_009128.1
5730525O22Rik	AK017789
Mtap7	NM_008635
Cdk6	NM_009873.2
Zzz3	NM_198416.2
Nfia	NM_177176.2
Maf	NM_001025577.2
Rbpj	NM_009035.4
B4galt5	NM_019835.2
Ccdc45	NM_177088.2
Dtnb	AK083752
Cd97	NM_011925.1
St6gal1	NM_145933.3
Pmp22	NM_008885.2
Clec2d	NM_053109.2

Luzp1	NM_024452.2
Grcc10	NM_013535.1
S100a6	NM_011313.2
Mrpl1	NM_053158.1
Ltbp1	AK054512
Klhdc4	NM_145605.1
Fasn	NM_007988.3
Tmem23	NM_144792.2
Nfkb2	NM_019408.1
Junb	NM_008416.1
Gpr68	NM_175493.2
9830002I17Rik	XM_126365.3
Spp1	NM_009263.1
2700038C09Rik	NM_025598.1
Rasgef1b	NM_181318.4
Prkcbp1	NM_027230.3
Med8	NM_020000.2
Usp7	NM_001003918.2
Tlr2	NM_011905.2
2310040G24Rik	XM_001480154.1
Fndc7	NM_177091.2
Clstn3	NM_153508.3
Slc4a4	NM_018760.1
Epb7.2	NM_013515.1
Nfyb	NM_010914.2
Lrrc33	NM_146069.4
Vav3	NM_020505.2
Ptafr	XM_357441.1
Mdfic	NM_175088.3
6330442E10Rik	NM_178745.3
Rapgef2	NM_001099624.1
Synpr	NM_028052.3
Il6ra	NM_010559.2
Phlpp	XM_129968.4
Reep3	NM_178606.4
Uchl4	NM_033607.1
Cdk5rap2	NM_145990.3
Taf5	NM_177342.3
2310045A20Rik	NM_172710.3
Papd4	NM_133905.1
Pi4kb	NM_175356.1
Wnt5a	NM_009524.2
Fer1l3	XM_001480162.1
Prss2	NM_009430.1
Cd9	NM_007657.2
Cebpd	NM_007679.4
Ralgds	NM_009058.1

Entpd1	NM_009848
Wsb1	NM_001042565.2
Snx29	NM_028964.3
Nfe2l2	NM_010902.3
Gas7	NM_008088.1
Scd3	NM_024450.2
Mrpl33	NM_025796.2
Socs3	NM_007707.2
Gabpb2	NM_029885.1
AB124611	NM_206536.1
Dtd1	NM_025314.1
Lox	NM_010728.1
6330503K22Rik	NM_182995.1
Cnot2	NM_028082.1
Icam2	NM_010494.1
Cxcl4	NM_019932.2
Ttpal	NM_029512.2
Plekho2	NM_153119.2
Dppa3	NM_139218.1
Asph	NM_133723.2
Stx7	NM_016797.4
Stard3nl	NM_024270.2
Eya3	NM_010166.2
Prrc1	NM_028447.2
Fhl3	AF114382
D930001I22Rik	NM_173397
2210010L05Rik	NM_133829.1
Syne2	NM_001005510.2
Abr	NM_198894.1
Creg1	NM_011804.2
Fcgr1	NM_010186.4
Il17rd	NM_134437.1
Cdc2l5	NM_001081058.1
Dpp3	NM_133803.1
Rhoc	NM_007484.1
Unc5b	NM_029770.2
5830415L20Rik	NM_001042501.1
Arhgef10l	NM_172415.2
Atp5g3	NM_175015.2
Adk	NM_134079.1
Timm13	NM_013895.2
Adss	NM_007422.2
Zc3h11a	NM_144530.5
Prx	NM_198048.1
Ngrn	NM_031375.3
Tmem120b	NM_001039723.2
Prkag2	NM_145401.1

Ppp3r1	NM_024459.2
Atp13a3	XM_001480958.1
Eif2ak4	AK077199
Pfkfb2	NM_008825.3
Acsl4	NM_001033600.1
Zubr1	XM_001479450.1
2310043N10Rik	NR_003513.2
Lass6	NM_172856.3
Coro6	NM_139128.1
Fkbp3	NM_013902.4
Ankrd55	NM_029898.2
Ddx50	NM_053183.1
Laptm4a	NM_008640.2
Lrrc28	NM_175124.4
Rara	NM_009024.2
Notch2	NM_010928.1
Rpl31	NM_053257.1
5031425E22Rik	XM_149592.1
Atp8b4	XM_141343
H2afx	NM_010436.2
Emg1	NM_013536.1
Ssfa2	NM_080558
2610204M08Rik	NM_198411.2
Akr1a4	NM_021473.2
Art3	NM_181728.1
E030010A14Rik	NM_183160.3
Galnt9	NM_198306.1
Abca17	NM_001031621.1
Actn1	NM_134156.1
Tacc1	NM_199323.2
Zc3h12a	NM_153159.1
Muc1	NM_013605.1
Cnpy3	NM_028065.2
Hivep2	NM_010437.2
Cc2d1b	NM_177045.2
Eif4a2	NM_013506
Cmah	NM_007717.1
Zeb2	NM_015753.3
Cux1	NM_009986.3
Npm3-ps1	NR_002702.1
C5ar1	NM_007577.3
Adap2	NM_172133.1
Smap2	NM_133716.2
Tle4	NM_011600.2
Col17a1	NM_007732.1
Hoxa1	NM_010449.3
AI836003	NM_177716.2

Hal	NM_010401.3
Usp6nl	NM_181399.3
Olfr726	NM_146316
Wdfy3	NM_172882
Myo3b	XM_194023.1
Erh	NM_007951.1
Rps19bp1	NM_175109.3
Btg2	NM_007570.2
Dcbld2	NM_028523.3
Ttc9	NM_001033149.2
Mrpl2	NM_025302.3
Birc6	AK086619
Itgb1	NM_010578.1
Nup160	NM_021512.2
Mnt	NM_010813.2
Emp1	NM_010128.4
Bcl7b	NM_009745.1
Nfatc4	NM_023699.2
Creb3l2	NM_178661.3
Gtf2e1	NM_028812.3
Ado	NM_001005419.1
Atp5l	NM_013795.4
3110003A17Rik	NM_028440.1
1700017N19Rik	XM_487140
Cltc	NM_001003908
Cdca3	NM_013538.4
Dhx35	NM_145742.1
Aldh9a1	NM_019993.3
Sall4	NM_175303.3
Tiprl	NM_145513.3
Bbc3	NM_133234.1
Jph1	NM_020604.1
Zfand3	NM_148926.2
Gdi2	NM_008112.4
Tex14	NM_031386.1
Nuak1	NM_001004363.1
Rffl	NM_001007465.1
Slc5a3	NM_017391.2
Cntnap5a	NM_001077425.1
Egr3	NM_018781
Gpatch4	NM_025663.2
Ggnbp1	NM_027544.1
Il20ra	NM_172786.1
2310005N03Rik	NM_025511.2
Opn5	NM_181753.2
8430432M10Rik	NM_176831.2
Gm826	NM_001033411.1

Lyzl4	NM_026915.2
1700012B15Rik	NM_028796
Col22a1	XM_907370.3
H2-Q1	NM_010390.2
Prnpip1	NM_080469.2
Mgat5	NM_145128.3
S1pr1	NM_007901.4
Lass2	NM_029789.1
Alad	NM_008525.3
Mppe1	NM_172630.1
Zc3h14	NM_029334.1
Ifnar2	NM_010509.1
Tmem119	NM_146162.1
St6galnac6	NM_001025311.1
Sepn1	NM_029100.2
Veph1	NM_145820.1
Dirc2	NM_153550.3
Gucy2g	NM_001081076.1
Tsen2	NM_199033.1
C130026I21Rik	NM_175219.3
Gsx1	NM_008178.2
Slc7a1	NM_007513.3
Txndc11	NM_134105.2
Plce1	NM_019588.2
1700112H15Rik	XM_149010.2
Tmem41b	NM_153525.5
Cdca1	NM_023284.1
Gm71	NM_001033236.2
AI427809	NM_001033454.1
Grk5	NM_018869.2
B3galnt1	NM_020026.2
Gda	AK044078
Cps1	NM_001080809.1
Esr1	NM_007956.4
5430405G24Rik	XM_152907.3
Hnt	NM_172290.3
March7	NM_020575.2
Nomo1	NM_153057.3
Sfpi1	NM_011355.1
Tm7sf3	NM_026281.2
1600014C10Rik	NM_028166.3
Brms1	NM_134155.1
Hells	NM_008234.3
Fndc3b	NM_173182.1
Axl	NM_009465.3
2810474O19Rik	NM_026054.2
6530418L21Rik	NM_175398.3

Phtf2	NM_172992.2
Leprel2	NM_013534.4
Cenpn	NM_028131.3
Tdrd7	NM_146142.1
Arid1a	NM_033566.1
Dusp14	NM_019819.3
Cic	NM_027882.2
Ifnab	NM_008336.2
1700027L20Rik	XM_906114.3
Cd19	NM_009844.2
Sf1	NM_011750.1
1700011E24Rik	XM_128789.5
Terf2ip	NM_020584.1
Ogg1	NM_010957.3
Tmem69	NM_177670.3
Casc1	NM_177222.3
Lpl	NM_008509.2
Gm572	NM_001085505.1
Itpkc	NM_181593.2
Crat	NM_007760.3
1700019M22Rik	NM_027076.2
Akna	NM_001045514.1
Fam176b	NM_172145.3
Itpr2	NM_010586.1
Slc7a14	NM_172861.2
Bcl2l1	NM_009743.4
BC006965	NM_146031
Cryga	NM_007774.3
Fli1	NM_008026.4
Selk	NM_019979.1
Clca5	NM_178697.4
Osbpl9	NM_173350.1
Lpcat3	NM_145130.1
1500035H01Rik	NM_023831.3
Zfp644	NM_026856.2
2310033K02Rik	NM_001080708.1
B3gat1	NM_029792.1
Nubpl	NM_029760.1
March8	NM_027920.4
Sergef	NM_013789.1
Slc30a6	NM_144798.4
3110023E09Rik	AK014071
Foxc1	NM_008592.2
Cln6	NM_001033175.1
D4Ertd196e	XM_975887.1
1810019J16Rik	NM_001083916.1
Ube2i	NM_011665.3

Clec4d	NM_010819.3
A430090L17Rik	NM_177004
Sspn	NM_010656.2
Pim1	NM_008842.3
Tatdn1	NM_175151.2
Uqcrc2	NM_025899.2
Lrp1	NM_008512.2
Tnrc4	NM_172434.2
Maz	XM_133827.2
Etv6	NM_007961.3
Arl6ip6	NM_022989.2
Atp8b2	NM_001081182.1
Cetn1	NM_007593.5
Iqch	XM_981814.1
Spink10	NM_177829.3
C230095G01Rik	NM_178768.3
Grin2b	NM_008171.3
Mmp9	NM_013599.2
Hsd11b1	NM_008288.1
Wipf2	NM_197940.1
Ncf4	NM_008677.1
4932443I19Rik	XM_980156.1
Dr1	NM_026106.4
C730024G19Rik	XM_921354.2
Tmc1	NM_028953.2
Eps8	NM_007945.2
Efna1	NM_010107.3
Ptp4a2	NM_008974.2
Pacrgl	NM_025755.3
4932425I24Rik	NM_001081025.1
Slc6a14	NM_020049.3
Arf2	NM_007477.4
Prr13	NM_025385.2
4930543E12Rik	XM_922816
Edem1	NM_138677.2
D1Ertd471e	NM_001164528
Nsl1	NM_198654.2
Rnf186	NM_025786.2
Ptchd3	XM_109751.6
Smg7	NM_001005507.1
Wdr36	NM_144863.3
BC017158	NM_145590.1
Gapdh	NM_008084.2
Rps20	NM_026147
Chmp4b	NM_029362.3
2310028O11Rik	XM_901483.3
Dip2c	NM_001081426.1

Igsf3	NM_207205.1
Pon1	NM_011134.2
OTTMUSG00000000997	NM_001037932.1
Fcer1g	NM_010185.2
Olfr951	NM_001011812.1
Cct5	NM_007637.2
Rps27a	NM_001033865.1
4930522O17Rik	XM_898933.3
Diap2	NM_017398.2
Fmnl1	NM_019679.2
1200011M11Rik	NM_024262.1
Bckdhb	NM_199195.1
Htr6	NM_021358.1
Galnt4	NM_015737.3
Olfr586	NM_147111.1
Scnn1a	NM_011324.1
Dpp10	NM_199021.2
Scg5	NM_009162.3
Exoc2	NM_025588.2
Elovl5	NM_134255.2
Trcg1	NM_001014398.1
Dlx1as	NR_002854.1
Tmem16b	NM_153589.1
Zfp364	NM_026406.2
Zc3h6	NM_178404.2
Grm4	NM_001013385.1
Ppp1r9b	NM_172261.2
Gh	NM_008117.2
Phospho1	NM_153104.2
4930546C10Rik	XM_484744
Sh3bgrl3	NM_080559.1
Zmat5	NM_026015.2
Cngb3	NM_013927.2
Hip1	NM_146001
2310011J03Rik	NM_025521.3
Rassf8	NM_027760.2
Arl4d	NM_031160.1
Morn4	NM_198108.2
Mef2d	NM_133665.3
Mgst3	NM_025569.1
Ctsd	NM_009983.2
Gm528	XM_986482.1
Bms1	NM_194339.1
Slc13a4	NM_172892.1
Bard1	NM_007525.2
Cd5l	NM_009690.1
Npffr1	XM_905368.1

Pltp	NM_011125.2
2610209M04Rik	NM_025665.1
1700010C24Rik	NM_027401.2
Ctss	NM_021281.1
Il6	NM_031168.1
Ubash3b	NM_176860.5
Pgs1	NM_133757.2
Ror1	NM_013845.4
Cyp26b1	NM_175475.2
Psma6	NM_011968.2
Kpna2	NM_010655.3
Myo16	NM_001081397.1
Mllt6	NM_139311.2
S100a7a	NM_199422.1
Sec22b	NM_011342.2
Gltp	NM_019821.2
Ssbp4	NM_133772.1
1600002D24Rik	XM_001473403.1
Gad2	NM_008078.1
Oxa1l	NM_026936.3
1600014C23Rik	XM_128667.1
Commd4	NM_025417.1
Frrs1	NM_009146.1
4933402G07Rik	AK016617
Chsy1	NM_001081163.1
Mnd1	NM_029797.1
Setdb1	NM_018877.2
Spry4	NM_011898.2
Mtx2	NM_016804.2
Prl4a1	NM_011165.3

Table S2. List of additional metabolic and regulatory genes (nCounter Panel Plus). The nCounter GX Human Immunology Kit v2 (Nanostring Technologies) was extended by probes for 30 additional genes.

Gene names	**Accession no.**
ABCA1	NM_005502.2
ABCG1	NM_207174.1
ACAT1	NM_000019.2
APOA1	NM_000039.1
APOE	NM_000041.2
BIRC2	NM_001166.3
BIRC3	NM_182962.1

CASP5	NM_004347.1
CETP	NM_000078.2
CYP27A1	NM_000784.3
CYP7A1	NM_000780.3
HSP90AA1	NM_001017963.2
HSP90AB1	NM_007355.2
HSP90B1	NM_003299.1
LPL	NM_000237.2
MAP3K7	NM_145333.1
NFKBIB	NM_002503.3
NLRC4	NM_021209.3
NLRP1	NM_033004.2
NR1H2	NM_007121.4
NR1H3	NM_005693.2
PLTP	NM_006227.2
PPARD	NM_006238.4
PSTPIP1	NM_003978.3
PYDC1	NM_152901.2
RIPK2	NM_003821.5
SCARB1	NM_005505.3
SLC2A4	NM_001042.2
SREBF1	NM_001005291.1
SUGT1	NM_006704.3

Table S3. LXR target gene list for GSEA analysis of human atherosclerotic plaques. The list contains direct LXR target genes identified in Pehkonen *et al. (54)* covered by the nCounter GX Human Immunology Kit v2 (Nanostring Technologies) and the 30 additional genes (Supplementary Table 2).

LXR target gene names	**Accession no.**
ABCA1	NM_005502.2
ABCG1	NM_207174.1
ABL1	NM_005157.3
BAX	NM_138761.3
BID	NM_001196.2
BIRC2	NM_001166.3
CCR6	NM_031409.2
CD276	NM_001024736.1
CD44	NM_001001392.1
CD58	NM_001779.2

CD82	NM_002231.3
CEBPB	NM_005194.2
DUSP4	NM_057158.2
EGR1	NM_001964.2
ETS1	NM_005238.3
FADD	NM_003824.2
GNLY	NM_006433.2
HLA-DRB1	NM_002124.2
HSP90AA1	NM_001017963.2
ICAM3	NM_002162.3
IL1RN	NM_000577.3
IRF5	NM_002200.3
ITGAX	NM_000887.3
LILRB3	NM_006864.2
MCL1	NM_021960.3
MIF	NM_002415.1
NFATC3	NM_004555.2
NLRP3	NM_001079821.2
NR1H3	NM_005693.2
PRDM1	NM_001198.3
PRKCD	NM_006254.3
PSMB8	NM_004159.4
PTGER4	NM_000958.2
RPL19	NM_000981.3
S100A8	NM_002964.3
S100A9	NM_002965.2
SDHA	NM_004168.1
SMAD5	NM_005903.5
STAT3	NM_139276.2
TGFBR2	NM_001024847.1
TNF	NM_000594.2
TRAF3	NM_145725.1
TUBB	NM_178014.2
XBP1	NM_005080.2

Table S4. Original data of all figures. Microsoft Excel spreadsheet including all original data and exact p-values of significant results.

Reviewer: 1

This is an impressive study investigating the regression of atherosclerosis by cyclodextrins. The authors demonstrate an LXR-dependent macrophage programming and production of 27OH in macrophages as well as reverse cholesterol transport and their effects on atherosclerosis regression.

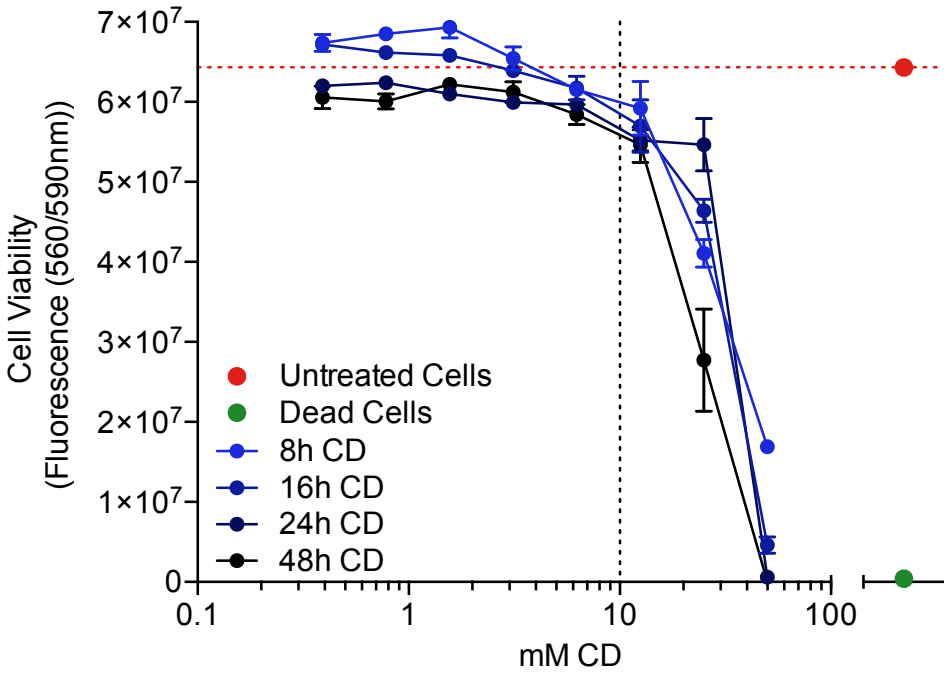
Thank you very much for your kind words of encouragement.

1. The authors typically use 10mM cyclodextrin with macrophages in their cell systems. In a buffer system this will typically be cytotoxic and cause major losses of cell protein. If used in serum, the toxicity may be less- but this is never made explicit anywhere in figures. The cyclodextrin will catalyze cholesterol and oxysterol movement from serum and to serum (see Atger at al ref 16), which will differ from the results in a buffer. This changes the interpretation of a number of figures- the authors should clarify the following:

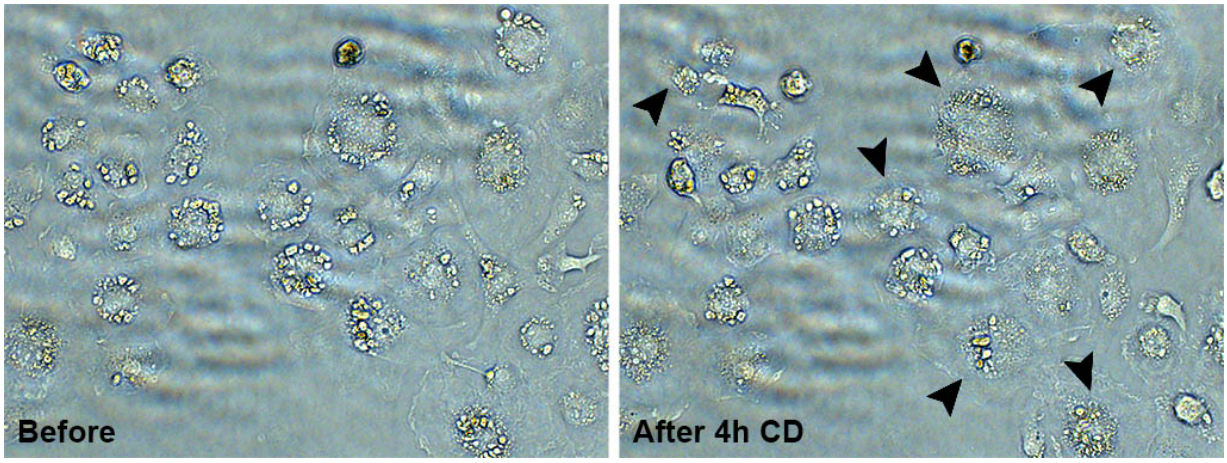
a. Fig 3F What is cell protein after 10mM cyclodextrin, and is the expression of crystal area reduced when expressed per cell protein?

b. Fig 4 B-H. Please clarify the incubation media into which cyclodextrin was added - PBS or HDL or serum- for each panel. In Materials and Methods (Analysis of crystal-derived cholesterol in macrophages) synthetic HDL was used with cyclodextrin to measure efflux- please indicate which figures this combination was used- the use of HDL with cyclodextrin is qualitatively and quantitatively different to the use of cyclodextrin alone- see Atger et al 16.

We would like to address these questions together in detail:
Regarding the CD toxicity in our cell stimulation system: We specifically chose 10 mM CD for our *in vitro* experiments because we had determined it to be below the toxicity threshold under the experimental conditions we have used in the studies reported in this manuscript. We have now incorporated viability assays of macrophages incubated with increasing CD concentrations into the revised manuscript as a new Supplementary Fig 3 (see also pasted in this letter below).

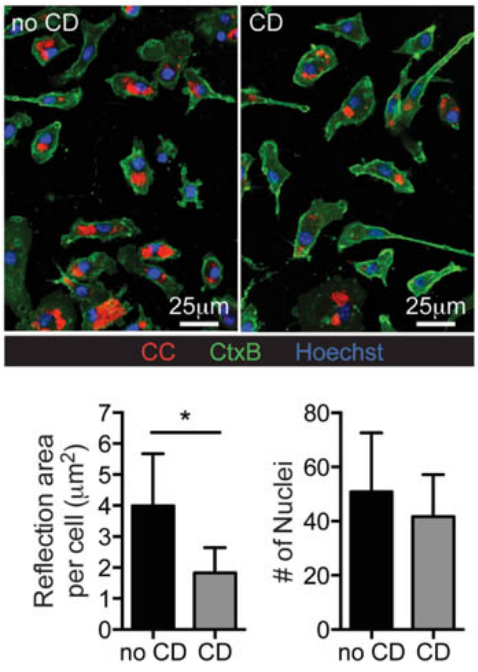


Regarding the protein levels for Fig. 3F: Protein levels were not measured in the experiments, as we have no indication that we loose cells during the treatment. As stated above, 10 mM CD does not affect viability of macrophages. Furthermore, the main purpose of Figure 3F is to illustrate that CD rapidly mediates intracellular CC dissolution, a finding that can be directly visualized in living cells. For the reviewer, we have provided screen captures from a simple light microscopic video acquisition of iMacs after incubation with CC (Before) and “After 4h CD” treatment. The images clearly highlight the rapid CD mediated intracellular CC dissolution. Of note, the video shows that the cells are still moving after 4 h of treatment, which is an indication that they remain viable throughout the observation period. We have also appended a video that documents this. This video is not part of the manuscript.



Regarding the dissolution of crystal area per cell: In the manuscript we opted to represent how CD mediates loss of total crystal area (Fig. 3F). After feeding of crystals the macrophage cultures were washed and no extracellular crystals remained visible. Hence, the total crystal area represents intracellular crystals.

As further evidence we have now also included confocal images and quantification of intracellular CC dissolution per cell after 24h of 10 mM CD incubation. This figure (pasted here below) is now Supplementary Fig. 4 in the revised manuscript.



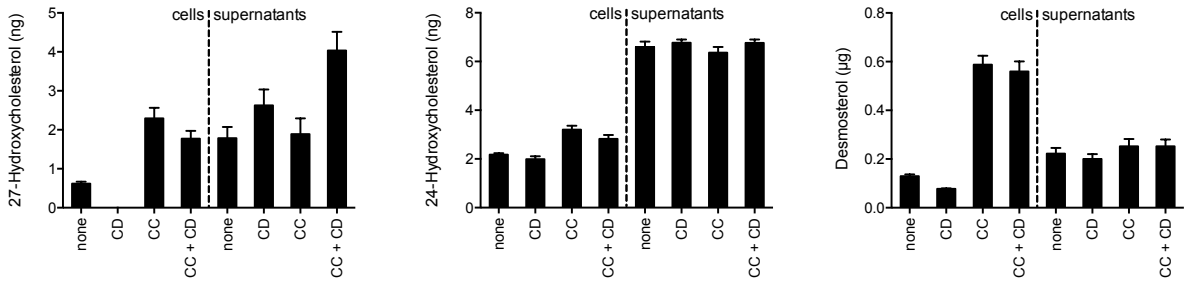
Regarding the medium used for these experiments: As stated in the Materials and Methods section: “Immortalized mouse macrophages from wild type C57BL/6 mice were cultured in DMEM supplemented with 10% fetal calf serum (FCS) and 10 µg / ml Ciprobay-

500 (Bayer)“.

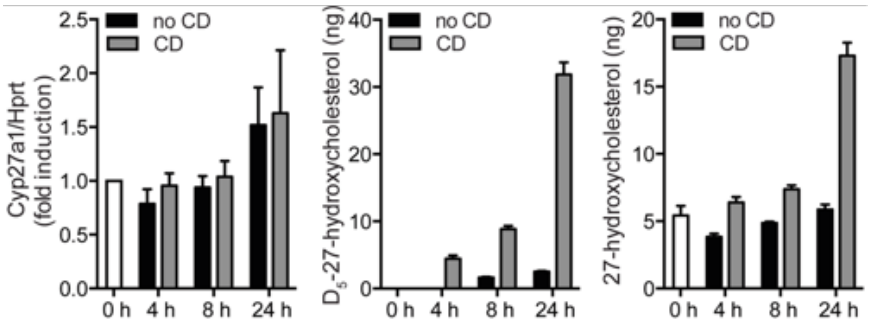
We apologize for the confusing statement in the “Analysis of crystal-derived cholesterol in macrophages”. The sentence referred to an experiment in a previous version of the manuscript, but none of the experiments shown in the current form include reconstituted HDL. As has been previously described *(1)*, HDL can augment the cholesterol efflux capacity of CD. We had performed an experiment confirming these results, but have excluded it from the current manuscript because we felt it did not contribute to the overall story or provided novel insight. The sentence has been removed from the materials section.

2. If cyclodextrin promotes the production of 27OH, can the authors report on the production of other oxysterols? What does cyclodextrin do to Cyp27A1 activity? Is 27OH relevant to the LXR effect? What happens if cyclodextrin is added to cells deficient in Cyp27A1? Are LXR pathways still activated?

Thank you very much for the interesting question. We have now also measured the production of other endogenous LXR agonists including 24-hydroxycholesterol and desmosterol in response to CC and CD incubation and found mainly 27-hydroxycholesterol to be affect by CD treatment. These data suggest that CD primarily influences the production of 27-hydroxycholesterol in macrophages. See data below.

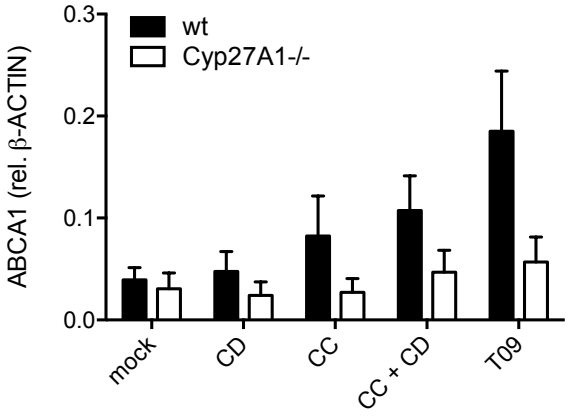


Interestingly, CD does not influence Cyp27A1 expression in CC-loaded BMDMs while oxysterols are produced in the same cells (see below). This implies that CD either provides more substrate for Cyp27A1 to the local site of oxysterol production or increases the rate of enzymatic production or both.



The atheroprotective effects of CD are most likely multifactorial and are not solely dependent on 27-hydroxycholesterol-mediated LXR agonism. In the manuscript we propose several potential pathways including increasing cholesterol solubility and cholesterol excretion, changed extrahepatic macrophage cholesterol metabolism and decreased local anti-inflammatory effects.

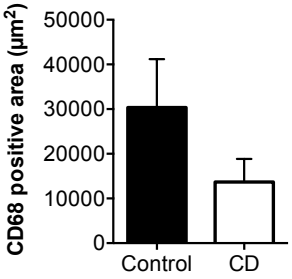
In response to the reviewer's suggestion, we have now investigated ABCA1 protein expression in Cyp27A1 knockout BMDM and found a decreased response to CD treatment (see below WB analysis of three individual mice per genotype - densitometric analysis, mean plus SEM). However, to discern the exact role of Cyp27A1 in this context goes beyond the scope of the manuscript, especially since the role of Cyp27A1 in atherogenesis is not fully understood and very complex due to different functions in different cell types *(2)*.



3. The number of macrophages in the plaque was unaffected by cyclodextrin yet inflammation was reduced as indicated by IL1 and by ROS (Figure 1 E-J). Please discuss non macrophage-mediated regulation of inflammation. Are there other examples of macrophage number being unaltered but plaque inflammation being reduced for comparison or is this a unique phenotype?

CD both decreases absolute macrophage numbers and has anti-inflammatory effects on

plaque macrophages. Figure 1F depicts CD68 positive staining area **in relation** to total plaque area. It is therefore a measure of relative plaque composition. But because total plaque area is reduced in CD treated mice, total CD68 positive staining i.e. absolute macrophage number is also reduced (see below).



Decreased inflammation can thus be attributed to a fewer absolute number of plaque macrophages. Additionally, CD has significant anti-inflammatory effects on human and murine atherosclerotic plaques Fig. 7-8 via LXR agonism. We have rephrased the results section to highlight this point more clearly.

Reviewer: 2

The manuscript by Zimmer et al explores the ability of cyclodextrin (CD) to dissolve cholesterol crystals and promote regression of atherosclerosis via LXRs. This is an interesting study that demonstrates that solubilizing cholesterol crystals promotes LXR ligand formation, reverse cholesterol transport via ABC transporters and a concomitant anti-inflammatory response to reduce plaque formation. They also showed that this is in part due to a reduction in ROS. Importantly the authors also show that this occurs in human plaques and that cholesterol excretion in urine can be demonstrated in humans taking CD. Overall is this is well-executed study and the conclusions drawn are largely consistent with the data. However, there are several areas in need of more explanation and some additional experiments that would help clarify the mechanism underlying the phenomena.

We greatly appreciate your constructive comments and thank you for the positive feedback.

One concern is that the CD is working through a pathway other than via the solubility of cholesterol crystals, oxysterol production and LXR activation. It appears that in some

cases there is a lack of association between a reduction in cholesterol crystals and a reduction in plaque area. In Figure 2D switching to chow diet reduced cholesterol crystals significantly but did not affect plaque area (compare Fig 2C control (red bar) with Fig 2D control (red bar). This suggests either a very steep threshold in the reduction of cholesterol crystals by CD to promote regression or that the CD treatment is doing something more that just reducing cholesterol crystals to reduce plaque formation. This needs to be explained.

The reviewer is entirely correct in their assumption that CD might be working through additional pathways other than the ones detailed in the manuscript. We have no data isolating a single specific mechanism. Indeed, it is likely that CD mediated atheroprotection is multifactorial. However, our data clearly demonstrate that CD promotes LXR activation in plaque macrophages and that LXR is required in myeloid cells for CD-mediated atheroprotection in mice.

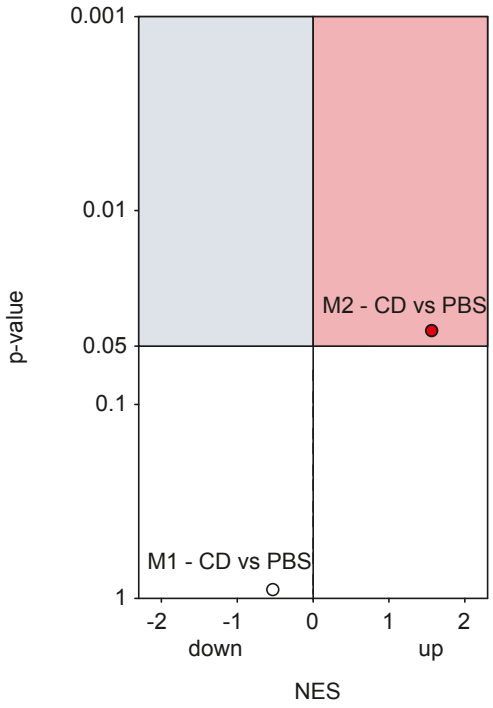
In the regression trial, switching to normal chow leads to a dramatic decrease in serum cholesterol levels. Because extracellular cholesterol crystal deposition in the subendothelial layer is primarily dependent on physical properties such as cholesterol concentration and solubility, this switch directly influences plaque composition in all mice. CD however has several beneficial properties that contribute to the regression of established plaques. First, it physically increases cholesterol solubility leading to rapid crystal dissolution and clearance. Second, it promotes cholesterol metabolism and efflux in macrophages. Third, it has anti-inflammatory effects. In contrast, local overabundance of “free” cholesterol upon crystal dissolution after chow change without CD treatment prompts foam cell formation, increased cell death and inflammation. We did not investigate plaque size at longer periods after the diet switch but a certain regression is possible.

We have discussed these limitations of our study in the revised version of the manuscript.

It would be also useful to probe if CD treatment of cholesterol crystal-loaded macrophages affects macrophage polarization. Work from several labs has shown that there is a change from M1 to M2 macrophages during plaque regression and it would be useful to examine whether this is the case in this model. Are M2 markers enhanced and M1 reduced upon CD treatment of macrophages in vitro (should be able to get this form the array data), and are M2 markers (e.g. Mannose receptor) increased in CD treated compared to control plaques by IHC?

Thank you for the very interesting suggestion. We have now investigated our data from

human atherosclerotic plaques and found a significant increase of M2 marker gene expression of CD treated samples compared to controls. We could not detect any relevant change in M1 marker genes (see data below). This data is in line with previous studies demonstrating only marginal M1-associated network patterns in M2-macrophages (*(3)*). The mannose receptor (MRC1), the scavenger receptors (MSR1 and SCARB1), DC-SIGN (CD209) and PPARγ were predominantly increased in CD-treated plaques. However these results need to be interpreted carefully. This is a complex and controversial topic as human M2 markers are not yet established in the literature. Additionally, our recent work suggests that the polarization of human macrophages are more complex than the traditional M1 and M2 dichotomous view suggests *(4-6)*. We therefore opted to not represent these data in the revised manuscript.



It is also unclear what is the point of photomicrograph in Figure 3E. A reduction in cholesterol crystals in the DC treated cells over time is difficult to discern from the image.

Figure 3E only demonstrates the rapid intracellular accumulation of CD. Cholesterol crystal dissolution was not quantified in this experiment. We have rephrased the manuscript to highlight this more clearly.

It is also puzzling in Figure 4F that ABCA1 expression is induced at the protein level by cholesterol crystal treatment alone (compare control lanes -/+ CC in the western blot). Could the authors please comment on this? Also it would be helpful to quantitate the expression of ABAC1 protein levels upon cholesterol crystal treatment as a function of CD treatment.

The fact that a cholesterol challenge induces ABCA1 expression is expected and congruent with the literature *(7, 8)*. It is the main mechanism by which macrophages clear excessive cholesterol and the foundation of reverse cholesterol transport *(9)*. As demonstrated by McConathy et al. *(10)* native macrophages can, to a certain extent, mediate cholesterol crystal dissolution and subsequently metabolize crystal derived excessive cholesterol. Figure 4F demonstrates that CD treatment enhances ABCA1 expression on top of these regulatory pathways. ABCA1 protein level as a function of CD treatment was quantified from three individual experiments (only the 10 mM dose is shown in densitometric analysis) and is depicted in a bar graph left of the representative Western blot. The figure legend has been updated to clarify this.

Overall this manuscript presents an interesting set of results that suggests CD therapy could have value in promoting regression of atherosclerosis.

Thank you very much.

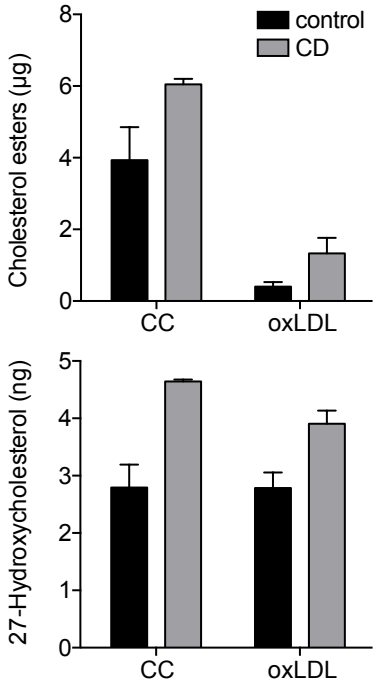
Reviewer: 3

The authors have demonstrated that 2-hydroyprpyl-β-cyclodextrin (CD) is a potential therapeutic for the treatment of atherosclerotic cardiovascular disease and have provided some insight into how CD is modulating macrophages to promote efflux and anti-inflammatory phenotype. There are however some issues still to be resolved.

1. Not all the cholesterol in atherosclerotic lesions and macrophages are in crystals. Yet only cholesterol crystals were used in the in vitro studies. The authors have previously shown that cholesterol crystals damage the lysosomes in macrophages and it is not clear if the amount of cholesterol taken up by the macrophages when cells are incubated with 100-200 ug/ml yields levels seen in macrophages in vivo. CD-mediated loss of cholesterol, induction of 27-hydroxysterol and modulation of LXR and anti-inflammatory gene expression in macrophages loaded with cholesterol via other means should be included.

This is a very intriguing question. In histological analyses of both human and mouse model atherosclerotic plaques (see Figure 1D) it is evident that the amount of cholesterol crystals far exceeds the number of macrophages. Yet, for most of our *in vitro* experiments we incubated macrophages with CC for only 3h. This is below the saturation threshold of macrophage crystal uptake and at the dose used does not induce significant lysosomal damage or NLRP3 inflammasome activation. We would like to point out that we completely concur with the reviewer's comment that one can hardly compare *in vitro* stimulated macrophages with the *in vivo* situation where macrophages are exposed to various forms of cholesterol and other danger signals. In fact, this was a main reason why we performed the *ex vivo* CD incubation experiments of human atherosclerotic plaques illustrated in Figure 7. Here we show that CD mediates cholesterol efflux into the supernatant (Fig. 7A), 27-hydroxycholesterol formation (Fig. 7B), LXR activation (Fig. 7F) and anti-inflammatory gene regulation (Fig. 7E, G, H). These plaques contain the macrophage/crystal ratio relevant to human atherosclerosis and should yield the "correct" levels of other potential confounders.

Still, foam cells formation is classically induced through oxLDL incubation *in vitro*. Here we provided data for the reviewer from one such experiment:



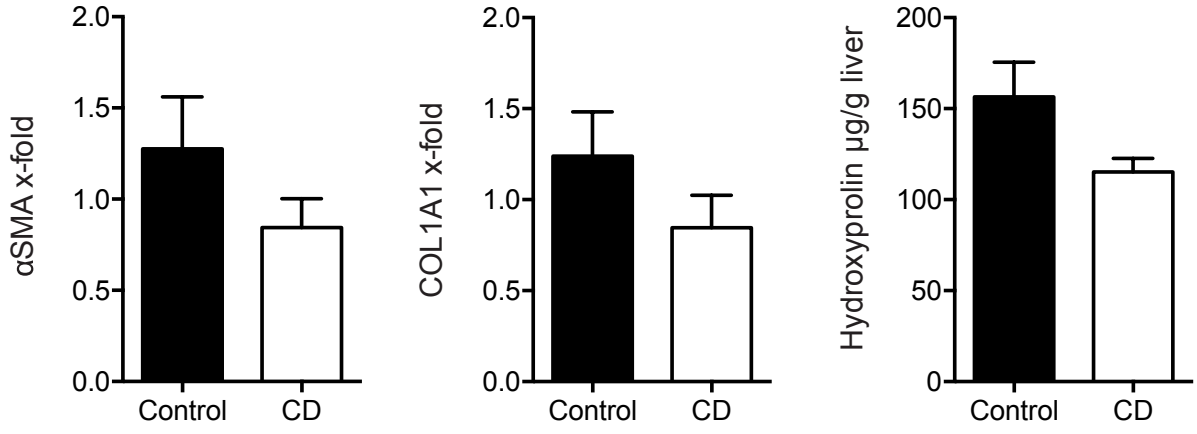
CD mediates cholesterol metabolism and 27-hydroxycholetserol production to a similar degree in both CC and oxLDL loaded macrophages. While this data supports our results

obtained with CC mediated foam cells, we opted not to add other means of cholesterol loading in the manuscript. We believe that our *ex vivo* human plaque studies are more relevant to the *in vivo* situation than experiments with mouse or human *ex vivo* generated foam cells.

2. Is there evidence of hepatic toxicity by the CD treatment (histological or ATL/AST measurements)? This is important preclinical information. The lack of toxicity in the NPC1 clinical trial could be related to the heightened state of liver toxicity in these individuals prior to treatment.

CD is not only used as a direct therapeutic agent in NPC1 patients but is also used as a complexing agent to increase the aqueous solubility of active substances and to increase their bioavailability and to improve stability. In addition, CD is used to reduce or prevent gastrointestinal and ocular irritation, reduce or eliminate unpleasant smells or tastes, prevent drug-drug or drug-additive interactions within a formulation, or to convert oils and liquid drugs into microcrystalline or amorphous powders *(11)*. In this context, CD toxicity has been thoroughly evaluated and is considered safe by both the FDA and EMA *(12, 13)*. Subjects receiving up to 24 g of CD per day over 15 days in clinical trials were free from side effects. In fact, Sporanox – a parenteral itraconazole formulation – contains 8 g of CD as a solvent and is given twice daily to patients with systemic fungal infections, no specific adverse effects have been described.

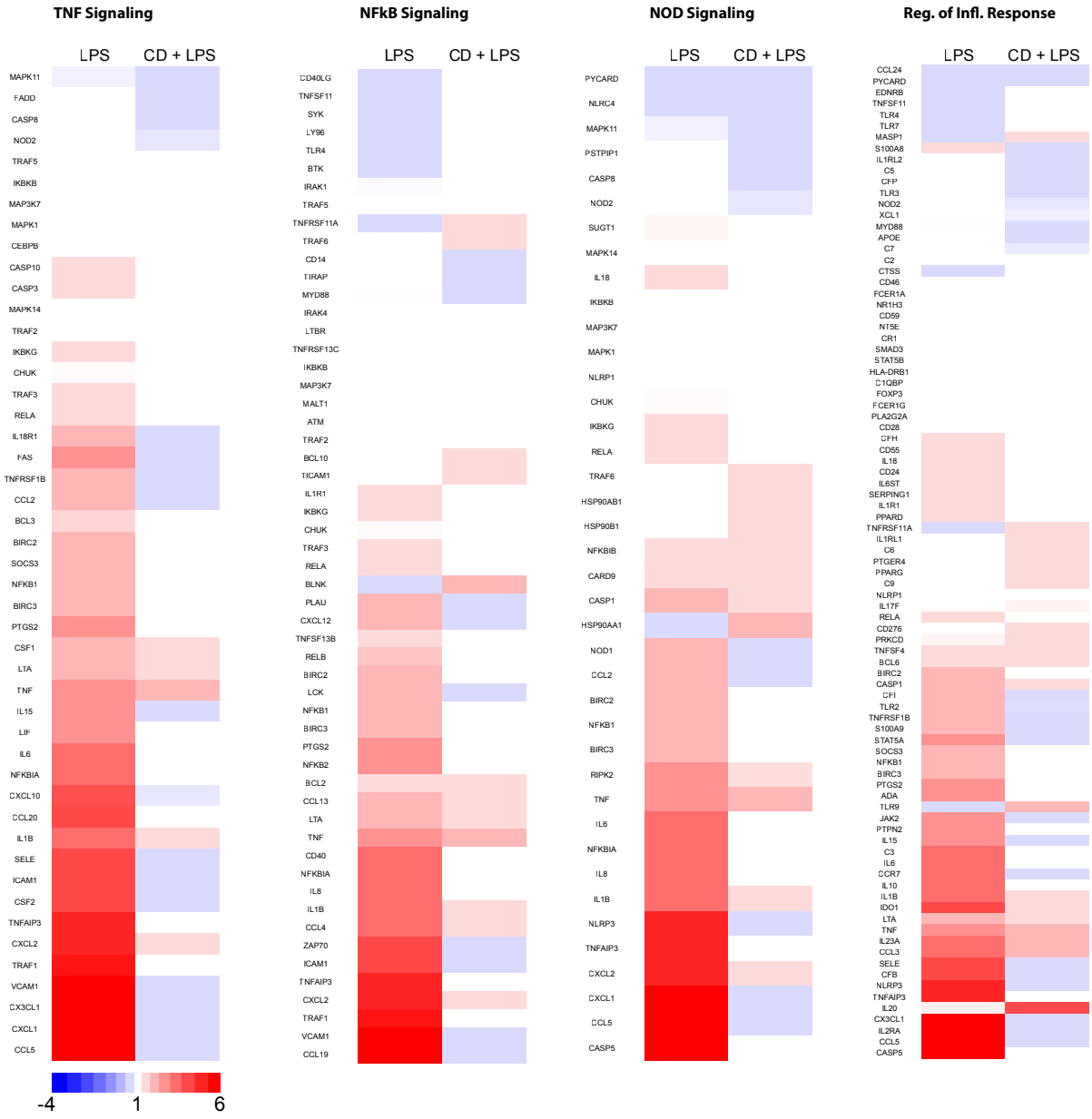
Nevertheless, we have now evaluated the livers of our CD and control treated mice for signs of hepatic toxicity. Interestingly, the signs of liver damage/fibrosis usually observed in ApoE-/- mice under cholesterol-rich diets *(14)* were found to be reduced upon CD treatment. This demonstrated that CD has liver protective rather than liver toxic effects in our experimental settings.



3. Why were the human carotid plaques treated with the strong proinflammatory stimulator LPS for 6 hours prior to the RNA and lipid analysis? This analysis no longer represents the state of the lesion in vivo. This may not have impacted on the lipid analysis where the only large difference was in the production of 27-hydroxycholesterol. This treatment is problematic for the gene expression analysis as it may have overestimated the effect of CD on inflammation and inflammasome components within the atherosclerotic lesion. The effect of CD treatment on the inflammatory genes is not as dramatic in the mouse lesions which were not exposed to LPS ex vivo.

We completely agree with the reviewer, addition of LPS to the specimens alters their inflammatory state and would make interpretations difficult. Yet, none of the data presented in the manuscript included samples treated with LPS. We have to apologize that we referred to LPS treated samples in the Material and Method section. We had indeed performed experiments, in which LPS treated plaque samples had been tested side by side, but these data were not shown. We apologize for having overlooked this detail. We have removed all mentions of LPS in the Material and Method section now.

For the information of the reviewer: CD treatment of LPS treated human plaques can indeed reduce a range of inflammatory reactions of the plaques to LPS (see below), yet as mentioned by the reviewer, the relevance of these findings for atherosclerosis are unclear and therefore we have not shown this dataset.



4. The aortic root, aortic arch and ascending aorta are all different segments of the aorta with different rates of lesion formation. The description in the Methods section (page10) is very unclear as to which location was analyzed for atherosclerosis. Also were the multiple sections quantitated adjacent sections or were they separated by some distance? The later provides a better assessment of the lesion area. How was it determined which sections to use to quantitate lesions at the different sites?

All plaques were assessed from section of the aortic root at midlevel of the aortic valve. We apologize for the confusing nomenclature; we have corrected this in the revised manuscript. Adjacent sections were used for the individual analyses but plaque size variability within the aortic root is far smaller than the observed differences between CD and control mice.

5. Is the quantitation in Fig 1B and analyses in Figs 1E-J the aortic arch or the aortic root (the aortic root images are shown in Fig. 1D)? If all the analysis is of the arch, then images of these vessels with the crystal should be shown. For figure 2 also, please indicate the lesion site analyzed. Did CD treatment equally affect all the arterial sites examined?

As described above and in the materials sections of the manuscript, for Fig. 1B-F sections of the aortic root at midlevel of the aortic valve were analyzed. Quantification of vascular ROS production (Fig. 1G) was performed in aortic segments of the proximal descending aorta just distal the ostium of the left subclavian artery. Cytokine levels illustrated in Fig. 1H-J were measure in plasma.

6. In Methods (page 12) it is stated that HDL was used in some efflux studies with the D6-cholesterol crystals. These studies were not included in the manuscript but would be useful to include as they would provide an estimate of how effective CD is in the presence of cholesterol acceptors.

We apologize for the confusing statement in the “Analysis of crystal-derived cholesterol in macrophages”. The sentence referred to an experiment in a previous version of the manuscript, but none of the experiments shown in the current form include reconstituted HDL. As has been previously described *(1)*, HDL can augment the cholesterol efflux capacity of CD. We had performed an experiment confirming these results, but have excluded it from the current manuscript because we felt it did not contribute to the overall story or provided novel insight. The sentence has been removed from the materials section.

Editor:

Thank you very much the detailed analysis of our manuscript. We have corrected all points you raised and would like to address your specific comments individually:

Page 2: Please avoid direct claims of novelty.

Direct claims of novelty have been removed from the main text.

Page 3: Whenever you say something is "significant," please provide p value(s) in the text.

References to significance have been removed from the main text. P-values are now detailed in Table S4 in the Supplementary Materials together with the original data of all figures.

Page 4: Please change to a complete sentence.

All subtitles have been changed to complete sentences.

"Feeding" implies some sort of animals that have mouths, not single cells. Please use a more appropriate term.

The term “feeding macrophages” has been changed to “loading macrophages with”.

Page 5: Our journal's style is not to use the abbreviations i.e. and e.g.

The abbreviation i.e. has been removed from the text.

Why does this figure not have any error bars or significance testing? Was it only done with n=1?

Figure 6B,C does not have error bars because it represents a calculated AUC from pooled samples of mice within a group. The volume of repetitive urine samples from individual mice is too small for GC-MS-SIM. The figure legend and Materials and Methods section have been reworded to describe this more clearly.

Page 6: Notably - The difference is so slight, even if significant, that I would hardly call it notable.

The term “notably” has been substituted with “Comparable to our findings in murine macrophages” to highlight the parallels between our murine *in vitro* and our human plaque *ex vivo* studies.

The table title just calls them "additional genes." Please be more specific with the title.

We have adapted the title of Table S2.

Protein names should always be in all caps and regular font, but gene and mRNA names should be in italics, with all caps if they are human and only first letter capitalized if they are murine. Please make sure to apply this rule throughout the text and figures, main and supplementary.

Gene and protein names within the main text and figures, as well as in the supplementary documentation have been change to the correct format.

Page 7: Please add a paragraph discussing the limitations of your study. This is our standard requirement for all articles.

A paragraph discussing the limitation of our study has now been included in the revised manuscript.

Page 9: Please begin with a Study Design paragraph (see checklist for instructions).

A short Study Design paragraph has been added to the beginning of the Materials and Methods section.

Please specify the vehicle in at least one place, and preferably whenever you discuss it.

For all animal experiments 200 μl 0.9 % NaCl solution was used the vehicle control. This has been specified accordingly in the revised manuscript.

Sacrificed - By what method?

Mice were sedated with an intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (16 mg/kg) and then sacrificed by cervical dislocation. This has been included in the new manuscript.

Page 10: KHB - Please specify ingredients or source company or provide citation.

The modified Krebs-HEPES buffer (pH 7.4) is composed of NaCl 99.01 mM, KCl 4.69 mM, $CaCl_2$ 1.87 mM, $MgSO_4$ 1.20 mM, Na-HEPES 20.0 mM, K_2HPO_4 1.03 mM, $NaHCO_3$ 25.0 mM, and D(+)glucose 11.1 mM and contained additional ascorbic acid (0.28 mM) and indomethacin (0.01 mM). This detail and a reference have been included in the manuscript.

Page 14: Written consent - Is the "and" a typo or did you mean "written and oral"?

This was indeed a typo, we have correct it in the revised manuscript.

Page 15: Please be more specific.

The paragraph on “Culturing of human atherosclerotic carotid plaques” has been rephrased to explain the experiment more clearly.

Page 33: For all composite figures such as bar graphs, please provide the original data, ideally in the form of a supplementary table in Excel format.

A supplementary table (Table S4) in Excel format has been included in the revised submission, it contains all the original data for the main and supplementary figures.

References

1. V. M. Atger, M. de la Llera Moya, G. W. Stoudt, W. V. Rodrigueza, M. C. Phillips, G. H. Rothblat, Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells, *J. Clin. Invest.* **99**, 773–780 (1997).

2. L. Zurkinden, C. Solcà, I. A. Vögeli, B. Vogt, D. Ackermann, S. K. Erickson, F. J. Frey, D. Sviridov, G. Escher, Effect of Cyp27A1 gene dosage on atherosclerosis development in ApoE-knockout mice, *FASEB J.* **28**, 1198–1209 (2014).

3. M. Beyer, M. R. Mallmann, J. Xue, A. Staratschek-Jox, D. Vorholt, W. Krebs, D. Sommer, J. Sander, C. Mertens, A. Nino-Castro, S. V. Schmidt, J. L. Schultze, A. Zirlik, Ed. High-resolution transcriptome of human macrophages, *PLoS ONE* **7**, e45466 (2012).

4. T. Rőszer, Understanding the Mysterious M2 Macrophage through Activation Markers and

Effector Mechanisms, *Mediators Inflamm.* **2015**, 816460 (2015).

5. J. Xue, S. V. Schmidt, J. Sander, A. Draffehn, W. Krebs, I. Quester, D. De Nardo, T. D. Gohel, M. Emde, L. Schmidleithner, H. Ganesan, A. Nino-Castro, M. R. Mallmann, L. Labzin, H. Theis, M. Kraut, M. Beyer, E. Latz, T. C. Freeman, T. Ulas, J. L. Schultze, Transcriptome-based network analysis reveals a spectrum model of human macrophage activation, *Immunity* **40**, 274–288 (2014).

6. J. L. Schultze, T. Freeman, D. A. Hume, E. Latz, A transcriptional perspective on human macrophage biology, *Semin. Immunol.* **27**, 44–50 (2015).

7. R. M. Lawn, D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, J. F. Oram, The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway, *J. Clin. Invest.* **104**, R25–31 (1999).

8. T. Langmann, J. Klucken, M. Reil, G. Liebisch, M. F. Luciani, G. Chimini, W. E. Kaminski, G. Schmitz, Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages, *Biochem. Biophys. Res. Commun.* **257**, 29–33 (1999).

9. K. J. Moore, F. J. Sheedy, E. A. Fisher, Macrophages in atherosclerosis:a dynamic balance, *Nature Reviews Immunology* **13**, 709–721 (2013).

10. W. J. McConathy, E. Koren, D. L. Stiers, Cholesterol crystal uptake and metabolism by P388D1 macrophages, *Atherosclerosis* **77**, 221–225 (1989).

11. European Medicines Agency, Background review for cyclodextrins used as excipients, *Committe for Human Medicinal Products*, 1–17 (2014).

12. S. Gould, R. C. Scott, 2-Hydroxypropyl-beta-cyclodextrin (HP-beta-CD): a toxicology review, *Food Chem. Toxicol.* **43**, 1451–1459 (2005).

13. T. Loftsson, P. Jarho, M. Másson, T. Järvinen, Cyclodextrins in drug delivery, *Expert Opin Drug Deliv* **2**, 335–351 (2005).

14. R. Schierwagen, L. Maybüchen, S. Zimmer, K. Hittatiya, C. Bäck, S. Klein, F. E. Uschner, W. Reul, P. Boor, G. Nickenig, C. P. Strassburg, C. Trautwein, J. Plat, D. Lütjohann, T. Sauerbruch, F. Tacke, J. Trebicka, Seven weeks of Western diet in apolipoprotein-E-deficient mice induce metabolic syndrome and non-alcoholic steatohepatitis with liver fibrosis, *Sci Rep* **5**, 12931 (2015).