

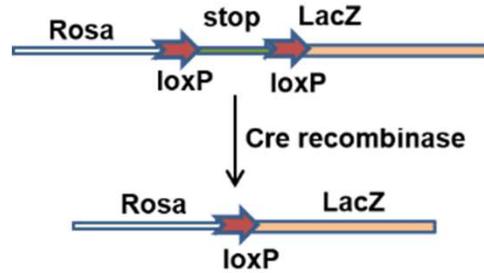
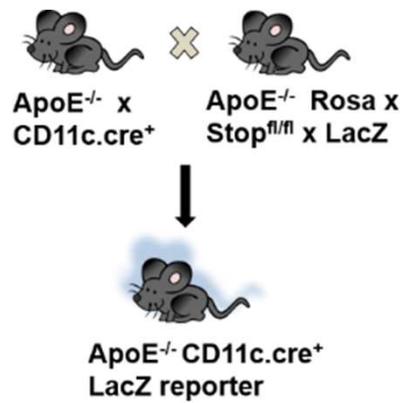
Supplemental information

Apolipoprotein E derived from CD11c⁺

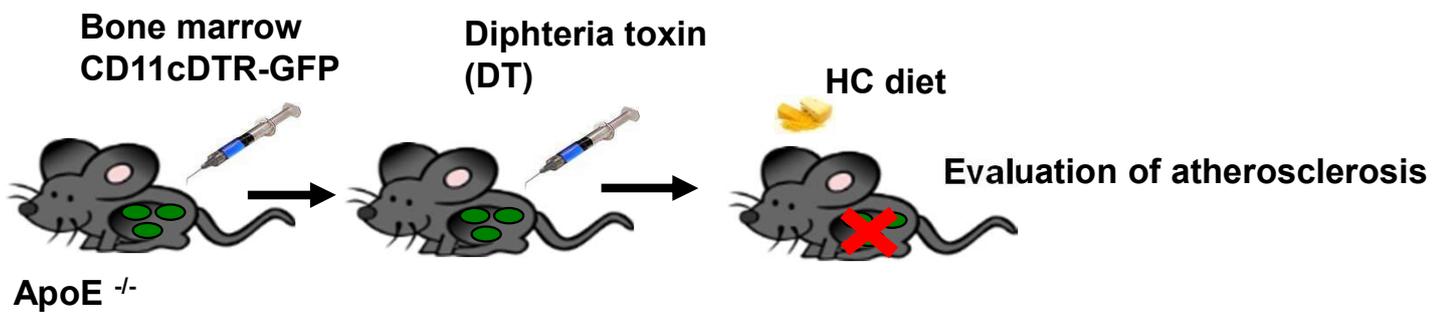
cells ameliorates atherosclerosis

Manuela Sauter, Reinhard J. Sauter, Henry Nording, Chaolan Lin, Marcus Olbrich, Stella Autenrieth, Christian Gleissner, Martin Thunemann, Nadia Otero, Esther Lutgens, Zouhair Aherrahrou, Dennis Wolf, Lars Zender, Sven Meuth, Robert Feil, and Harald F. Langer

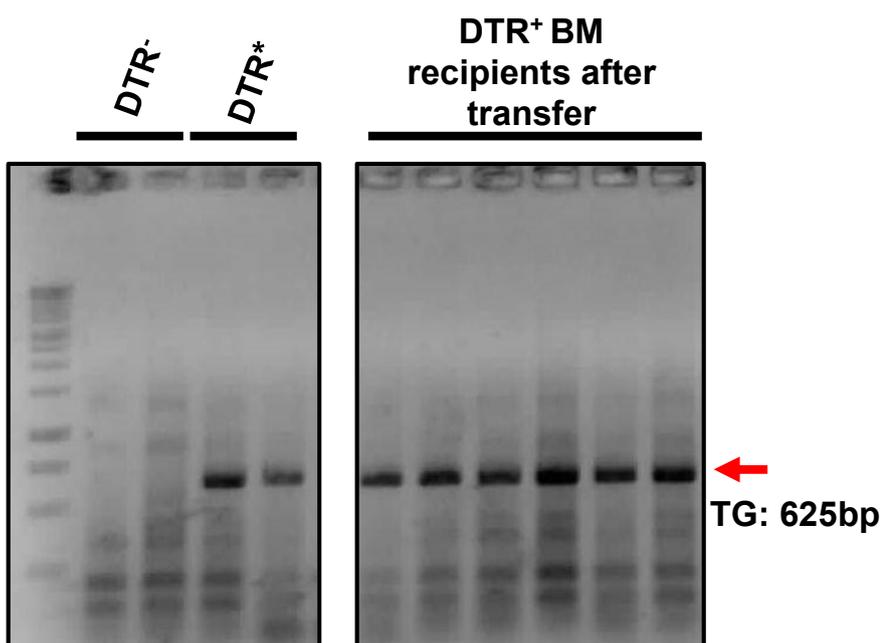
A



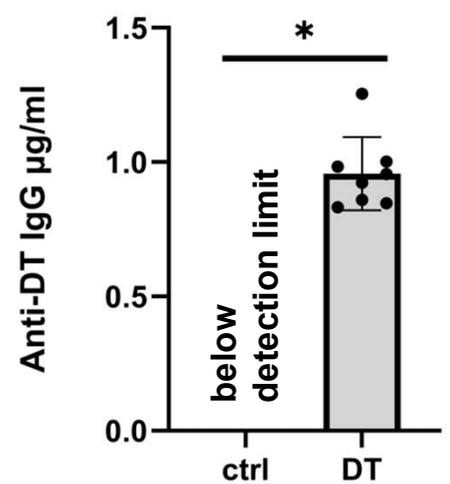
B

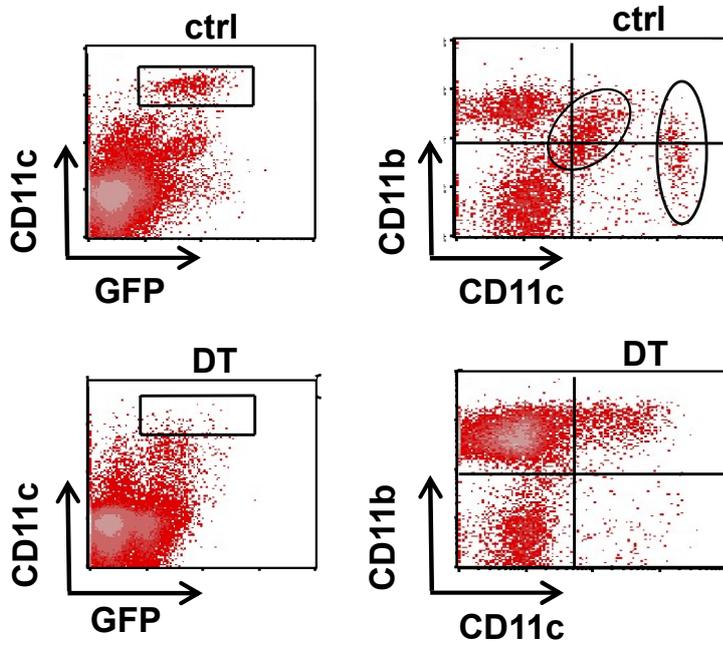
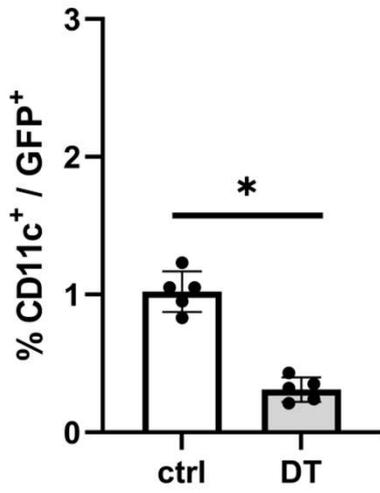
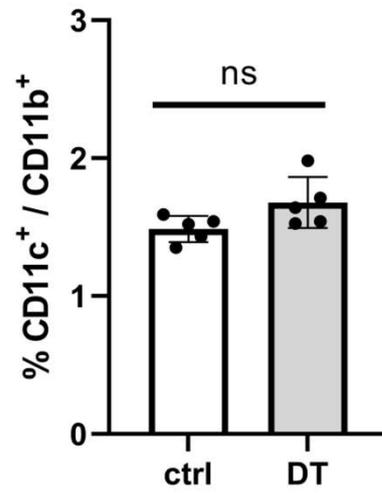
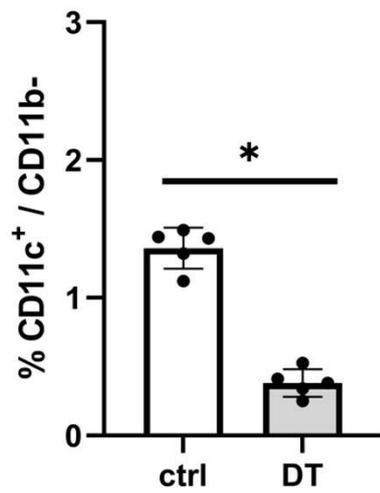
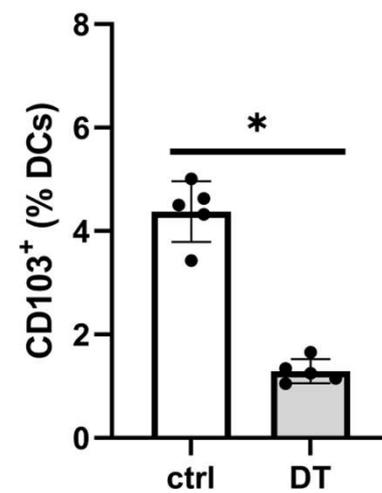


C

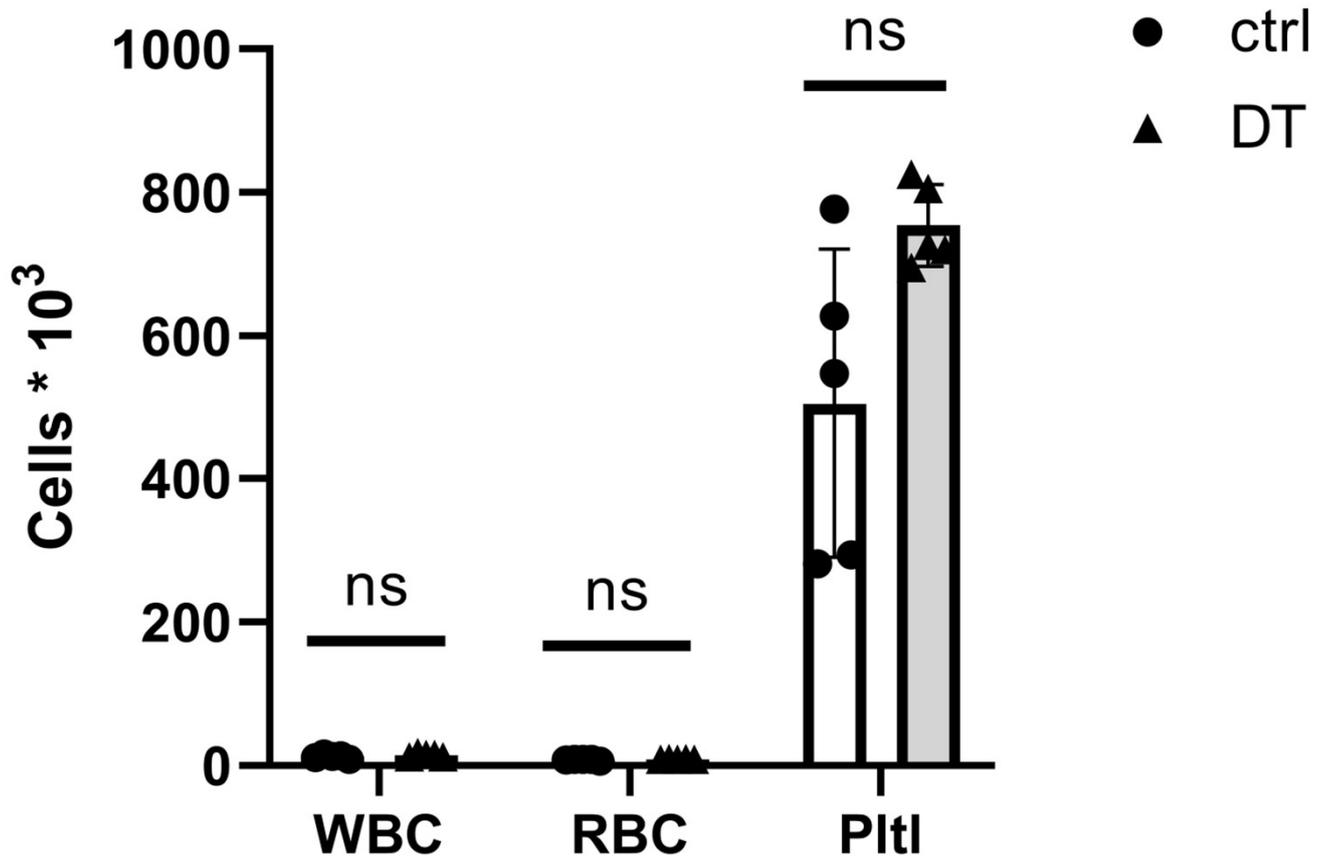


D

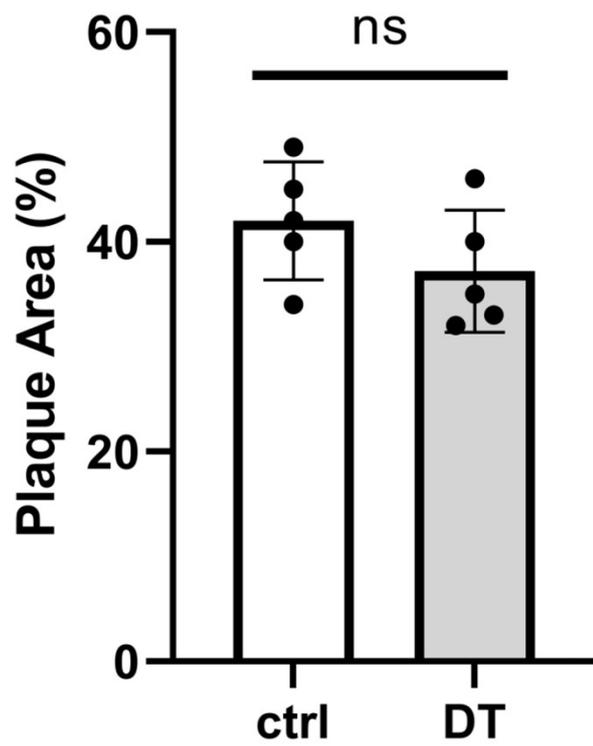


A**B****C****D****E**

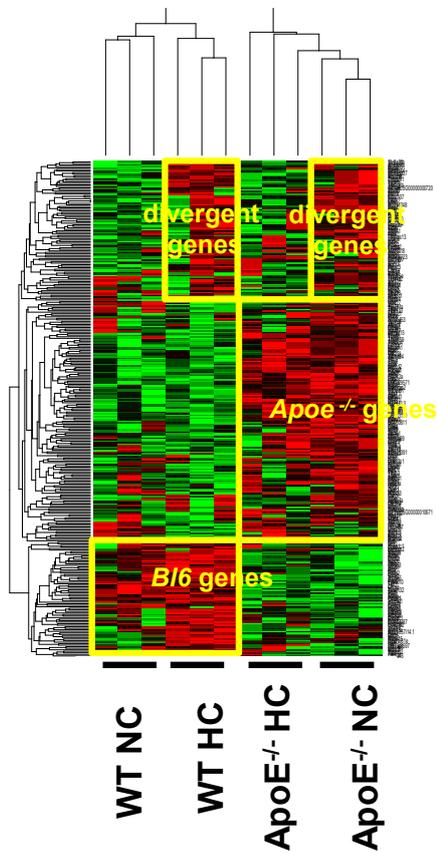
A



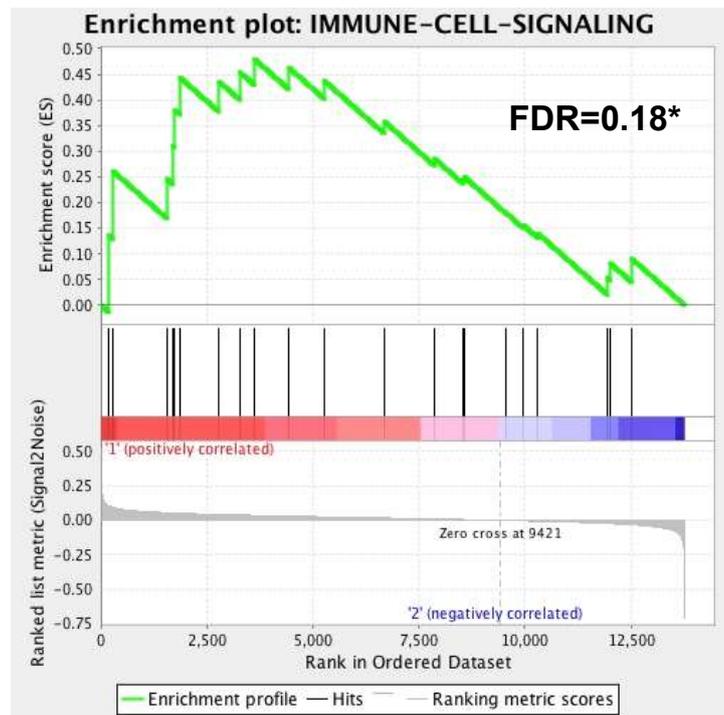
B



A

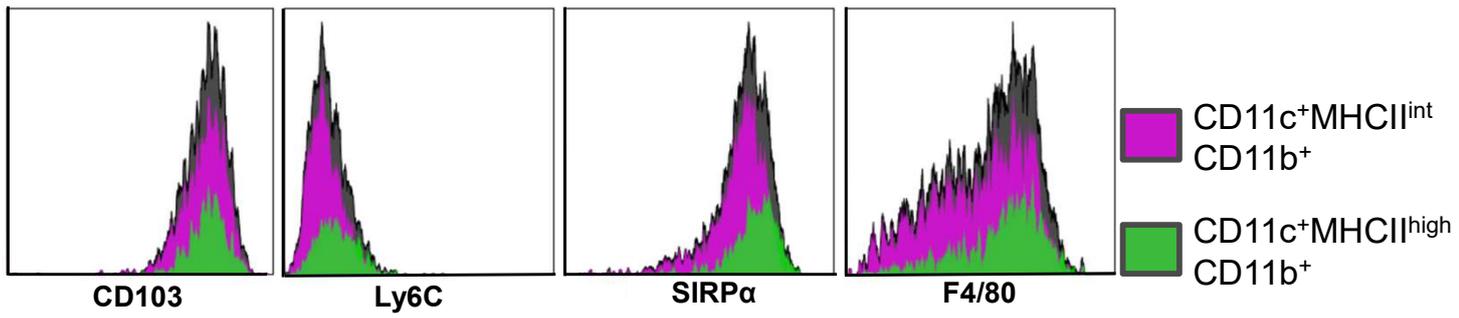
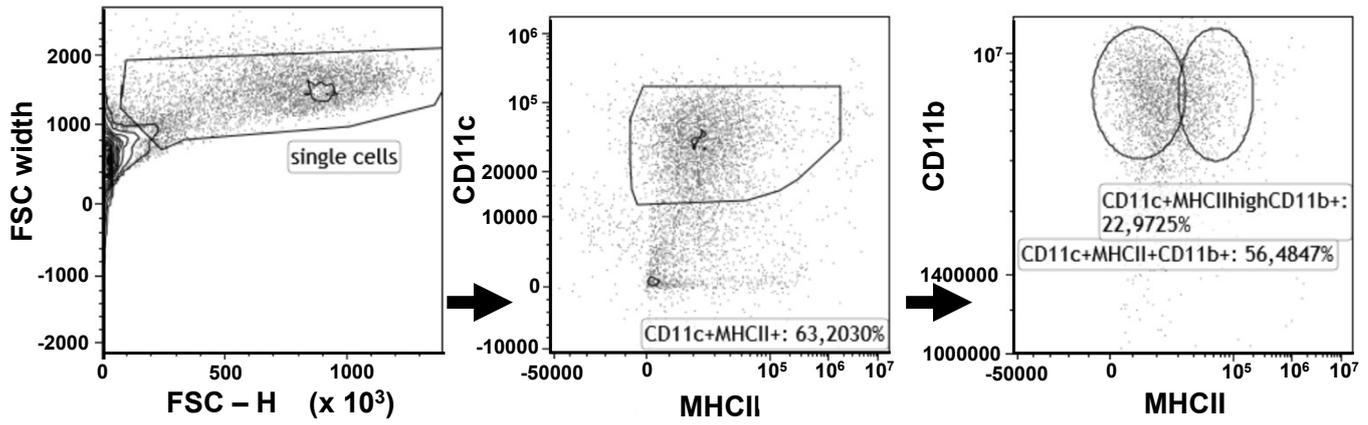


B



C

SampleName	ApoEHC1	ApoEHC2	ApoEHC3	B6NC1	B6NC2	B6NC3
APOA1	APOA1	apolipoprotein A-I				
PON1	PON1	paraoxonase 1				
LPL	LPL	lipoprotein lipase				
MSR1	MSR1	macrophage scavenger receptor 1				
NFKB1	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)				
IL6	IL6	interleukin 6 (interferon, beta 2)				
CSF1	CSF1	colony stimulating factor 1 (macrophage)				
SELE	SELE	selectin E (endothelial adhesion molecule 1)				
S100A8	S100A8	S100 calcium binding protein A8				
F3	F3	coagulation factor III (thromboplastin, tissue factor)				
IL1RN	IL1RN	interleukin 1 receptor antagonist				
COL18A1	COL18A1	collagen, type XVIII, alpha 1				
LCAT	LCAT	lecithin-cholesterol acyltransferase				
SREBF2	SREBF2	sterol regulatory element binding transcription factor 2				
VCAM1	VCAM1	vascular cell adhesion molecule 1				
ALOX12	ALOX12	arachidonate 12-lipoxygenase				
COL1A1	COL1A1	collagen, type I, alpha 1				
CD40	CD40	CD40 molecule, TNF receptor superfamily member 5				
MMP3	MMP3	matrix metalloproteinase 3 (stromelysin 1, progelatinase)				
TGFB1	TGFB1	transforming growth factor, beta 1 (Camurati-Engelmann disease)				
CLU	CLU	clusterin				



Legends to supplemental figures

Supplemental Figure 1 [Generation of mouse models for detection and long-term depletion of CD11c⁺ cells], Related to Figure 1. (A) Generation of a „CD11c reporter mouse“. ApoE^{-/-} mice were bred to CD11c^{cre+} and LacZ^{fl/fl} mice to obtain ApoE^{-/-} mice with their CD11c⁺ cells expressing β-Galactosidase. (B) Generation of a long-time depletion model for the ablation of CD11c⁺ cells. ApoE^{-/-} mice were irradiated twice (4 hours apart) with 6.5 Gy. 24h later, they received bone marrow of CD11c - diphtheria toxin receptor (DTR) -GFP mice retroorbitally. After a recovery period of 3 weeks, the animals were fed a HC diet and injected with diphtheria toxin (DT) twice weekly i.p. for a period of 6 weeks. (C) Blood was taken retroorbitally from ApoE^{-/-} mice 3 weeks after bone marrow (BM) transfer and screened for the DTR by PCR. DTR⁺: CD11c-DTR⁺ mice served as positive control. DTR⁻ mice served as negative controls. Shown is one representative image. (D) Measurement of anti-DT antibodies in BM chimeras after long-time depletion showed no significant detection of neutralizing antibodies in sera of DT treated animals. n=8 animals per group. *P<0.05. Irradiation and BM transfer was performed when mice were 6-8 weeks old. Data are the mean ± SD.

Supplemental Figure 2 [Characterisation of depleted cells in BM chimeras], Related to Figure 1. Bone marrow of CD11c.DTR-GFP mice was transferred into irradiated ApoE^{-/-} mice. After the depletion period of 6 weeks, single-cell suspensions were prepared from explanted spleens of BM chimeras and screened for CD11c, CD11b and GFP by flow cytometry. (A) A significant reduction of CD11c⁺/GFP⁺ and CD11c⁺/CD11b⁻ cells could be observed. (B-D) Further quantification showed that GFP⁺/CD11c⁺ and CD11c⁺/CD11b⁻ cells were depleted, while CD11c⁺/CD11b⁺ cells were not affected. The analysis further revealed that the depleted cells (CD11c⁺/CD11b⁻) were CD103⁺. n=5 animals per group. *P<0.05 vs ctrl. Data are the mean ± SD.

Supplemental Figure 3 [Blood cell analysis of BM chimeras], Related to Figure 1. (A) Blood cell analysis of retroorbitally taken blood of bone marrow chimeras showed no significant changes in cell counts of white (WBC) and red blood cells

(RBC) or platelets (Pitl). n=5 animals per group. *P<0.05. (B) Treatment with DT per se over a period of 6 weeks did not influence plaque development in ApoE^{-/-} mice compared to ctrl treated animals, as observed by Oil Red O staining of aortae. n=5 animals per group. *P<0.05. Data are the mean ± SD.

Supplemental Figure 4 [microarray analysis of BM derived CD11c⁺ cells of HC fed ApoE^{-/-} mice compared to WT], Related to Figure 2. mRNA of CD11c⁺ cells from spleens from WT and ApoE^{-/-} mice fed with standard or HC diet for 12 weeks was isolated. A microarray analysis was performed. (A) Heat map showing all differentially regulated genes with statistical significance (n = 3 animals per group. FDR <0.25, by LPE test). Gene expression was normalized and standardized. Red indicates high, and green indicates low gene expression. Genes and conditions were allowed to freely cluster in the y- and x-axes, respectively. Yellow boxes highlight gene clusters concomitantly or divergently upregulated in CD11c⁺ cells derived from WT or ApoE^{-/-} mice. (B) Enrichment plot of “immune cell signalling” gene set in HC fed ApoE^{-/-} compared to chow fed WT mice. All genes were ranked using the GSEA difference of class metric. For each gene in the gene set, the enrichment score was calculated and plotted against the position of the genes within the rank ordered data set. A significant enrichment was found (n = 3 animals per group, FDR <0.25, by LPE test). (C) Microarray gene expression as analyzed by gene set enrichment analysis (GSEA). Enrichment plot of the 21 genes mapped to the immune cell signaling pathway (WT on NC compared to ApoE^{-/-} mice on HC). Red indicates high, and blue indicates low gene expression.

Supplemental Figure 5 [Characterisation of BM derived CD11c⁺ cells by flow cytometry], Related to Figure 2. Bone marrow of wildtype mice was harvested and cells were cultivated in the presence of GM-CSF for 6 days. To characterize the cell culture, cultivated cells were analyzed by flow cytometry.

Table S1. [Primers used for genotyping of mice], Related to Key Resources Table, Oligonucleotides.

Oligonucleotides: Primers for genotyping		
Name	Source	Sequence
ApoE (oIMR0180)	eurofins	5'-GCC TAG CCG AGG GAG AGC CG-3'
ApoE (oIMR0181)	eurofins	5'-TGT GAC TTG GGA GCT CTG CAG C-3'
ApoE (oIMR0182)	eurofins	5'-GCC GCC CCG ACT GCA TCT-3'
ApoE flox for	eurofins	5'-GGC TTA GTG GGT AAA GGT GCT-3'
ApoE flox rev	eurofins	5'-GAC TAG GCA GGT GTG GAA TTA GA-3'
Alb CRE for	eurofins	5'-ACG ACC AAG TGA CAG CAA TG-3'
Alb CRE rev	eurofins	5'-CTC GAC CAG TTT AGT TAC CC-3'
CD11c cre (oIMR7841)	eurofins	5'-ACT TGG CAG CTG TCT CCA AG-3'
CD11c cre (oIMR7842)	eurofins	5'-GCG AAC ATC TTC AGG TTC TG-3'
CD11c cre (oIMR8744)	eurofins	5'-CAA ATG TTG CTT GTC TGG TG-3'
CD11c cre (oIMR8745)	eurofins	5'-GTC AGT CGA GTG CAC AGT TT-3'
DTR (oIMR1801)	eurofins	5'-GGG ACC ATG AAG CTG CTG CCG-3'
DTR (oIMR1802)	eurofins	5'-TCA GTG GGA ATT AGT CAT GCC-3'
DTR (oIMR8744)	eurofins	5'-CAA ATG TTG CTT GTC TGG TG-3'
DTR (oIMR8745)	eurofins	5'-GTC AGT CGA GTG CAC AGT TT-3'
LacZ (BB01)	eurofins	5'-CTC TGC TGC CTC CTG GCT TCT-3'
LacZ (BB02)	eurofins	5'-CGA GGC GGA TCA CAA GCA ATA-3'
LacZ (RF127)	eurofins	5'-GCG AAG AGT TTG TCC TCA ACC-3'