Supplemental Material

Extent and plaque composition of atherosclerotic lesions

Mice were anesthetized with isoflurane before sacrifice. Plasma cholesterol was measured using a commercial cholesterol kit (Sigma). The heart and aorta, including the brachiocephalic artery, were taken off, fixed in 4% paraformaldehyde for 2 hours. Lesion extent in the thoracic aorta represents the percentage of Oil red O staining. Necrotic cores were visualized using Masson’s Trichrome. The presence of macrophages and T cells within plaques were studied using respectively an anti-macrophages/macrophages antibody, MOMA-2 (EMD Millipore) and anti-CD3 (DAKO, Trappes, France). At least 4 sections per mouse were examined for each immunostaining, and appropriate negative controls were used. Terminal dUTP nick end-labeling (TUNEL; ApoDETEK kit, ENZO Diagnostics, DAKO) was considered as positive when at least 2 of 6 sections per mouse showed TUNEL staining. We performed morphometric studies using Histolab software (Microvisions).

Quantitative Real time PCR

Cells were lysed in detergent buffer RLT and spleens were lysed in Trizol and then subjected to RNA extraction and reverse transcription (Qiagen). Then, quantitative real-time PCR was performed on an ABI PRISM 7700 (Applied Biosystems) in triplicates. Cycle threshold for Gapdh (primers: Gapdh-R, 5′-CGTCCCGTAGACAAAATGTGGA-3′; Gapdh-L, 5′-GCGTGAGTGAGCTACAGGAAACA-3′) was used to normalize gene expression. Primers for Il10, Clec9a, Tgfβ, Il6, Il1 and Il1β are respectively: Il10 (primers: Il10-R, 5′-AAAGACAAAGCCAGAGTCCTTCAGAGAGAT-3′; Tgfβ (primers: Tgfβ-R: 5′CGGAGAGCTGGAATCCACTA-3′; Tgfβ-L, 5′-CCGCACACAGCAGTTCTCTCTC-3′), Il6 (primers Il6-R: 5′-GGTCTTGCTCCTTAGCCACTCTCTGT-3′; Il6-L, 5′-AAAGACAAAGCCAGAGTCTTCTCTCTAGAGAT-3′), Il1 (primers Il1-R, 5′-AGCAACAGCAAGGCGAAAA-3′; Il1-L, 5′-CTGGACCTGTGGGTTGTTGAG-3′), Clec9a (primers: Clec9a-R, 5′-TTTGGCAACATACAGCACAGCAG-3′; Clec9a-L, 5′-TGTGACTGCTCCCACAACTGG-3′), Il1b (primers Il1b-R: 5′GGGTGTGCCGTCTTTCATTA-3′; Il1b-L: 5′-GAAGAGCCCATCTCTGTGA-3′).

PCR conditions were 10 min at 95°C; 35 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s; and a final extension at 72°C for 20s.

Flow cytometry

Cell surface staining are the following: APC-conjugated CD45.1 (A20), FITC-conjugated CD45.2 (104), PE-Cy7 conjugated CD11c (HL3), Alexa fluor 700-conjugated CD8 (53-6.7), PE-conjugated F4/80 (BM8) (BD Biosciences), and eFluor 450-conjugated CD62L (clone MEL-14), APC-conjugated CD44 (IM7) (eBiosciences), Percp-Cy5.5-conjugated GR-1 (RB6-8C5), APC-conjugated CD25 (PC61.5) (eBiosciences), and V-500-conjugated B220 (RA3-6B2). Intracellular staining of forkhead box P3 (PE-Cy7-conjugated FOXP3) (FJK-16s) (eBiosciences) was performed. Splenocytes were stimulated in vitro for 4 h using leukocyte activation cocktail (BD) for intracellular staining. Briefly, cells were stained for surface markers followed by fixation and permeabilization using a kit (eBiosciences) for intracellular staining. Then, cells were stained with APC-conjugated II10 (clone JESS5-16E3) (eBiosciences). For CD8α+DCs staining, we used anti-CD11c-PE-Cy7 (clone: N418, Biolegend), MHC II Pacific blue (clone: M5/114.15.2, Biolegend), CD8a BV786 (clone: 53-6.7, Biolegend) and CD11b A700 (clone: M1/70, BDBiosciences).

Samples were acquired using a flow cytometer (LSRFortessa, Becton Dickinson) and data was analyzed using FlowJo software (TreeStar, OR, USA). Cell doublets were excluded using forward (FSC) and side (SSC) light scattering area (A) vs. width (W).
Supplementary Figure I DNGR1 deletion effects in atherosclerosis. A plasma cholesterol in Ldlr⁻/⁻ mice reconstituted with either control (Clec9a⁺/+ → Ldlr⁻/⁻) or Clec9a⁻/⁻ (Clec9a⁻/⁻ → Ldlr⁻/⁻) bone marrow after 5 weeks (control (n=7) and Clec9a⁺/+ (n=8)) or 7 weeks (control (n=12) and Clec9a⁺/+ (n=16)) or 13 weeks (control (n=8) and Clec9a⁻/⁻ (n=9)) of high fat diet (HFD). B plasma cholesterol C representative pictures and quantification of plaque size in aortic sinus of Apoe⁻/- (n=9) and Apoe⁻/-Clec9a⁻/⁻ (n=9) mice fed with HFD during 6 weeks D representative pictures and quantification of necrotic cores in plaques of Ldlr⁻/⁻ mice reconstituted with either (control (n=8) and Clec9a⁺/+ (n=9)) after 13 weeks of HFD. Mean values ± SEM are shown. *P<0.05, **P<0.001, ***P<0.0001.
Supplementary Figure II High levels of cholesterol decreases DNGR1 expression. A mRNA expression of Clec9a in spleens of male Ldlr⁻/⁻ mice on chow diet (CD) (n=3) or male Ldlr⁻/⁻ mice reconstituted with either control (Clec9a⁺/⁻ -> Ldlr⁻/⁻) or Clec9a⁻/⁻ (Clec9a⁻/⁻ -> Ldlr⁻/⁻) bone marrow after 7 weeks (control (n=6) or Clec9a⁻/⁻ (n=8)) or 13 weeks (control (n=7) or Clec9a⁻/⁻ (n=7)) of high fat diet (HFD). B Representative examples and quantitative analysis of flow cytometry-based GFP expression in splenocytes from either control (Clec9a⁺/⁻, GFP⁻) or Clec9a⁻/⁻ (GFP⁺) (see materials and methods). Splenocytes were incubated in absence (NS) or in presence of cyclodextrin-cholesterol at different concentrations (5, 10, 25, 50, 100 ug/ml) during 16 h. One representative experiment out of two is shown. Mean values ± SEM are shown. * P<0.05, **P<0.001, ***P<0.0001.
Supplementary Figure III. DNMR1 deletion in bone marrow protects against atherosclerosis in mice bearing Mfge8 deletion with accumulation of apoptotic/necrotic cells. A-B Representative photomicrographs and quantifications of TUNEL staining in lethally-irradiated male Ldlr−/− mice reconstituted with bone marrow from wild type control (n=4) or Clec9a−/− (n=5) or Mfge8−/− (n=7) or Mfge8−/−Clec9a−/− (n=15) mice. Percent lesion area occupied by acellular (apoptotic/necrotic) material are shown. Results were obtained after 4 weeks of recovery from bone marrow transplantation followed by 7 weeks on a high fat diet (HFD). C-D Representative photomicrographs and quantification of lesion size in Ldlr−/− mice reconstituted with either Control (n=4) and Clec9a−/− (n=5) or Mfge8−/− (n=7) or Mfge8−/−Clec9a−/− (n=15) bone marrow after 7 weeks of HFD. Results were obtained after 4 weeks of recovery from bone marrow transplantation followed by 7 weeks on a HFD. Mean values ± SEM are shown. *P<0.05, **P<0.001, ***P<0.0001.
Supplementary Figure IV DNGR1 effects on macrophage infiltration within plaques and on gene expression of inflammatory factors. **A** Representative photomicrographs and quantitative analysis of lesional macrophage infiltration in Ldlr−/− mice reconstituted with either Control (n=7) and Clec9a+/− (n=8) after 5 weeks of high fat diet (HFD), or either Control (n=12) and Clec9a−/− (n=16) after 7 weeks of HFD, or either Control (n=8) and Clec9a−/− (n=9) after 13 weeks of HFD. **B** DNGR1 deletion has no effect on mRNA of Il-6, Ifn-γ, Il-1β in spleens of Ldlr−/− reconstituted with either control or Clec9a−/− bone marrow after 7 weeks of HFD. Mean values ± SEM are shown. *p<0.05,**P<0.001, ***P<0.0001.
Supplementary Figure V DNGR1 deletion has anti-inflammatory effects at 5 weeks but not at 13 weeks of HFD. A-B DNGR1 deletion effects on il-10 and tgf-β mRNA in spleens of Ldlr⁻/⁻ mice reconstituted with either control (Clec9a⁺/+ → Ldlr⁻/⁻) or Clec9a⁻/⁻ (Clec9a⁻/⁻ → Ldlr⁻/⁻) bone marrow after 5 weeks (control (n=7) and Clec9a⁻/⁻ (n=8)) or 13 weeks (control (n=8) and Clec9a⁻/⁻ (n=9)) of high fat diet (HFD). C DNGR1 deletion increases IL-10 production in supernatants of splenocytes from Apoe⁻/⁻ (n=5) and Apoe⁻/⁻Clec9a⁻/⁻ (n=5) at 20 weeks-old after stimulation with LPS (1ug/ml) during 48 h. * P<0.05, **P<0.001, ***P<0.0001.
Supplementary Figure VI Constitution of control and Clec9α−/−CD8α+DC chimeric mice. A Frequency of DCs (CD11chighMHC IIhigh) and CD8α+DCs in the spleen of Cd11cCre−/Irf8flox/flox and Cd11cCre+/Irf8flox/flox analysed by flow cytometry (n=3/group). B Donor origin as defined by flow cytometry analysis of CD45.1% and CD45.2% of different cell types in spleens of chimeric Ldlr−/− mice transplanted with bone marrow mix (8:2) from Cd11cCre+/Irf8flox/flox (CD45.2) and control (CD45.1) mice (n=7). C Representation and quantification of CD8α+DC by flow cytometry in control group (CD8α+DC-Cont, n=7) or DNGR1-deficient CD8α+DC group (CD8α+DC-Clec9α−/−, n=8) after 7 weeks of HFD. Mean values ± SEM are shown. ***P<0.0001, *P≤0.05.
**Supplementary Figure VII effects of DNGR1 deletion in CD8α+DCs.**

A photomicrographs and quantitative analysis of lesional macrophages in Ldlr⁻/⁻ mice reconstituted with either control group (CD8α⁺DC-Cont, n=7) or Clec9a-deficient CD8α⁺DC group (CD8α⁺DC-Clec9a⁻/⁻, n=8) after 7 weeks of high fat diet (HFD).

B No change in activated (CD44⁺) and naive (CD62L⁺) spleen CD8⁺ T cells in the Clec9a-deficient CD8α⁺DCs (n=8) compared to control-CD8α⁺DCs (n=7) groups of mice.

C Il-10 mRNA in pooled Clec9a-deficient CD8α⁺DCs (n=3) or CD8α⁺DCs (n=3) compared to control-CD8α⁺DCs (n=3) or CD8α⁺DCs (n=3) isolated from chimeric Ldlr⁻/⁻ mice transplanted with bone marrow mix (2:8) from control (CD45.1) (n=7) or Clec9a⁻/⁻ (n=8) bone marrow cells mixed with Cd11cCre⁺/Irf8floxflox (CD45.2) bone marrow. Mean values ± SEM are shown.

*p<0.05, ***<0.0001.
Supplementary Figure VIII DNGR1 deletion in CD8α+DCs increases IL-10 expression in aorta. A Representative examples of flow cytometry-based GFP expression in purified CD45 cells from aorta of either control (Apoe<sup>−/−</sup> Clec9α<sup>−/−</sup> GFP−) or Apoe<sup>−/−</sup> Clec9α<sup>−/−</sup> (GFP+) (see materials and methods). B mRNA of il10, tnfa, il6 and tgfβ in aorta from the DNGR1-deficient CD8α+DCs (n=8) compared to control-CD8α+DCs (n=7) groups of mice. Mean values ± SEM are shown. **P<0.001.
Supplementary Figure IX DNGR1 deletion in CD8α+DCs increases IL-10 production by T cells.

A IL-10 production by anti-CD3 (5ug/ml) and anti-CD28 (1 ug/ml) stimulated spleen TCD3+ cells from Il-10+/+ mice, co-cultured in presence of LPS (1ug/ml)-treated spleen CD8α+DCs from Il-10+/+ or Il-10−/− mice, measured after 48 h of stimulation.

B-D IL-10, IL-17 and IFN-γ production by anti-CD3 (5ug/ml) and anti-CD28 (1 ug/ml) stimulated spleen CD3+CD8α+DCs from Apoe−/− or Apoe−/−Clec9a−/− mice (n=3), co-cultured in presence of LPS (1ug/ml)-treated spleen CD8α+DCs from Apoe−/− mice (n=3) or Apoe−/−Clec9a−/− mice (n=3), measured after 48 h of stimulation. Both CD8α+DCs and T cells were isolated from Apoe−/− mice (n=3) or Apoe−/−Clec9a−/− mice (n=3) after 20 weeks of chow diet. Mean values ± SEM are shown. *p<0.05, **P<0.001, ***P<0.0001.
Supplementary Figure X DNGR1 deletion in CD8α+DCs increases IL-10 production by T cells. A Representative and quantification by flow cytometry of regulatory T cell (CD4+CD25+Foxp3+) B IL-10 production by anti-CD3 (5µg/ml) and anti-CD28 (1 µg/ml) stimulated spleen CD4+ cells from wild-type (WT) mice, co-cultured in presence of LPS (1µg/ml)-treated spleen CD8α+DCs from chimeric Ldlr<sup>-/-</sup> mice transplanted with 2:8 mix of bone marrow from control (n=8) or Clec9a<sup>-/-</sup> (n=9) or Il-10<sup>-/-</sup> (n=8) or Clec9a<sup>-/-</sup>Il-10<sup>-/-</sup> (n=9) bone marrow cells mixed with Cd11c<sup>Cre<sup>/Irf8<sup>flox/flox</sup> bone marrow, after 7 wks of HFD and IL-10 was measured after 48 h of stimulation. C IL-10 production by anti-CD3 (5µg/ml) and anti-CD28 (1 µg/ml) stimulated spleen CD4+ cells from WT mice, co-cultured in presence of LPS (1µg/ml)-treated spleen CD8α+DCs from chimeric Ldlr<sup>-/-</sup> mice transplanted with 2:8 mix of bone marrow from control (n=8) or Clec9a<sup>-/-</sup> (n=9) in presence of control IgG or anti-IL-10 receptor neutralizing antibody (anti-IL-10r Ab, 10µg/ml). Mean values ± SEM are shown. *p<0.05, **P<0.001, ***P<0.0001.