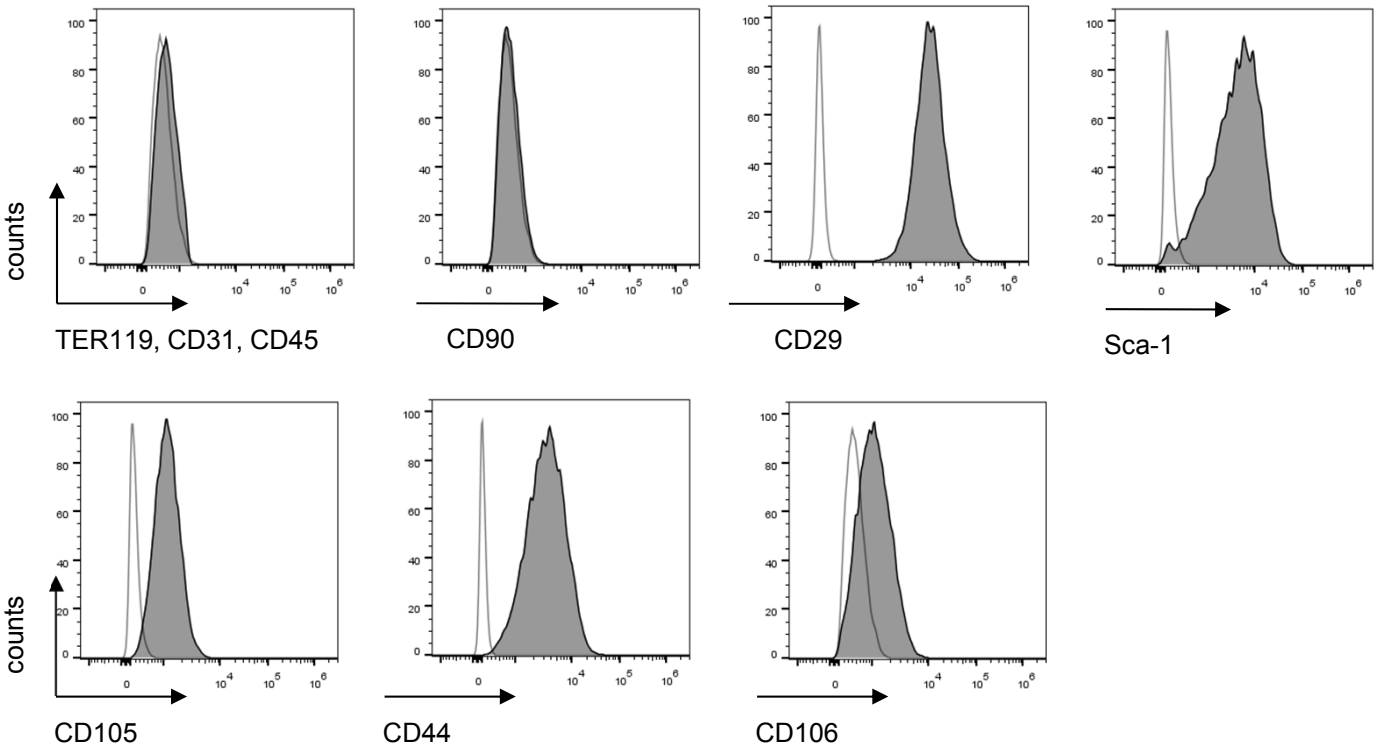
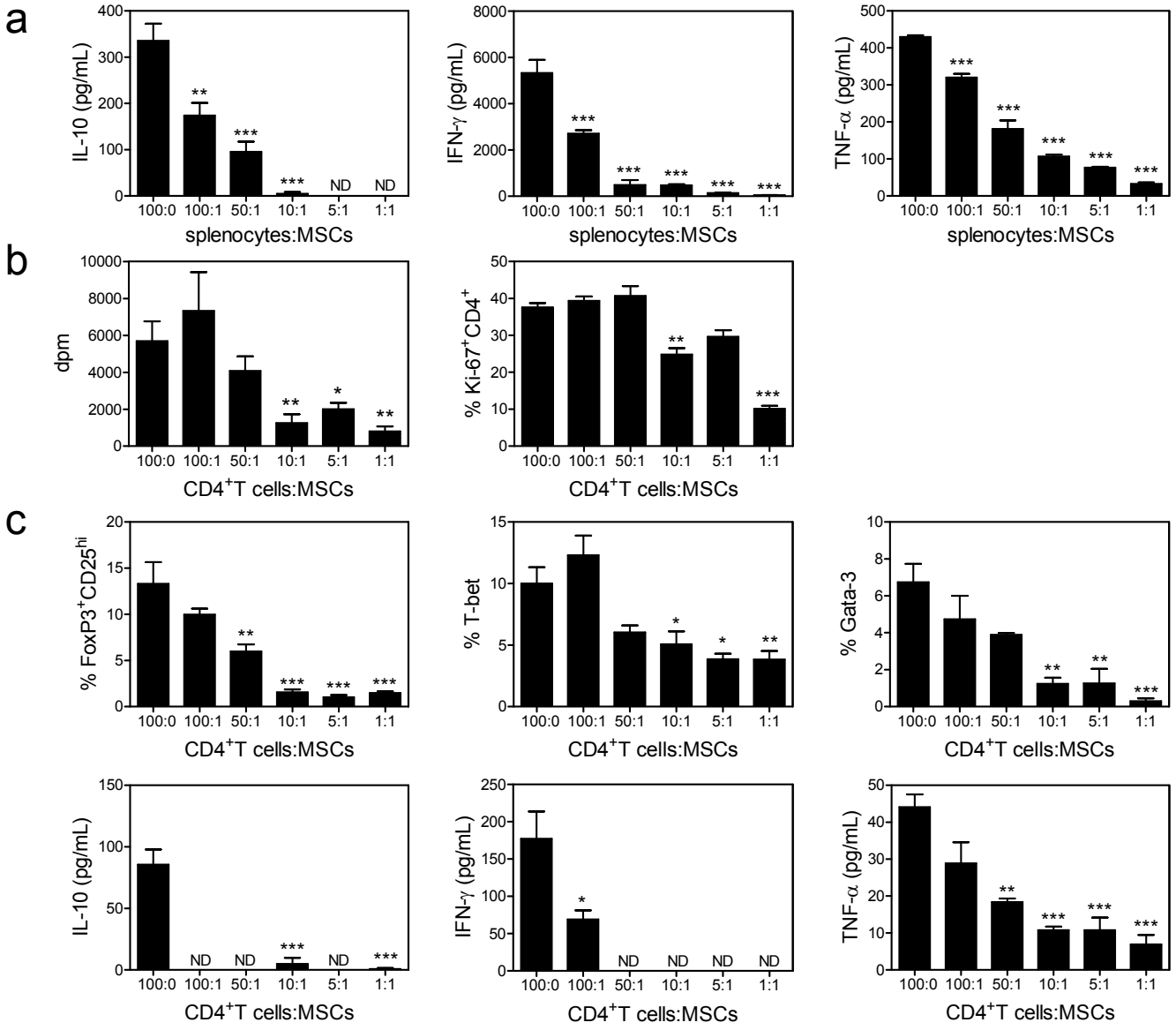
# Supplementary Information

## Mesenchymal Stem Cells Reduce Murine Atherosclerosis Development

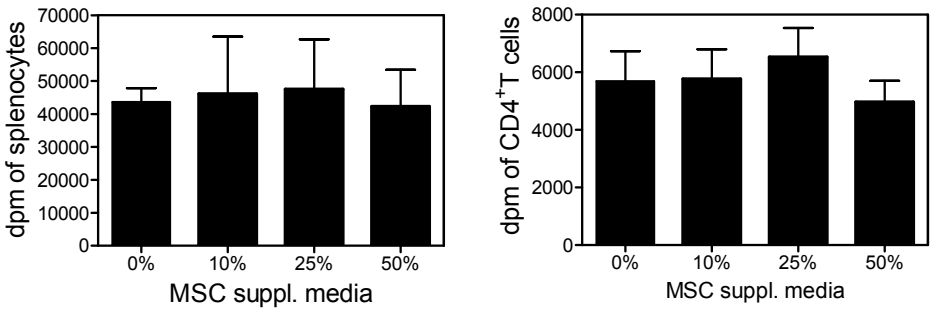
**Frodermann V, van Duijn J, van Pel M, van Santbrink PJ, Bot I, Kuiper J, de Jager SCA**



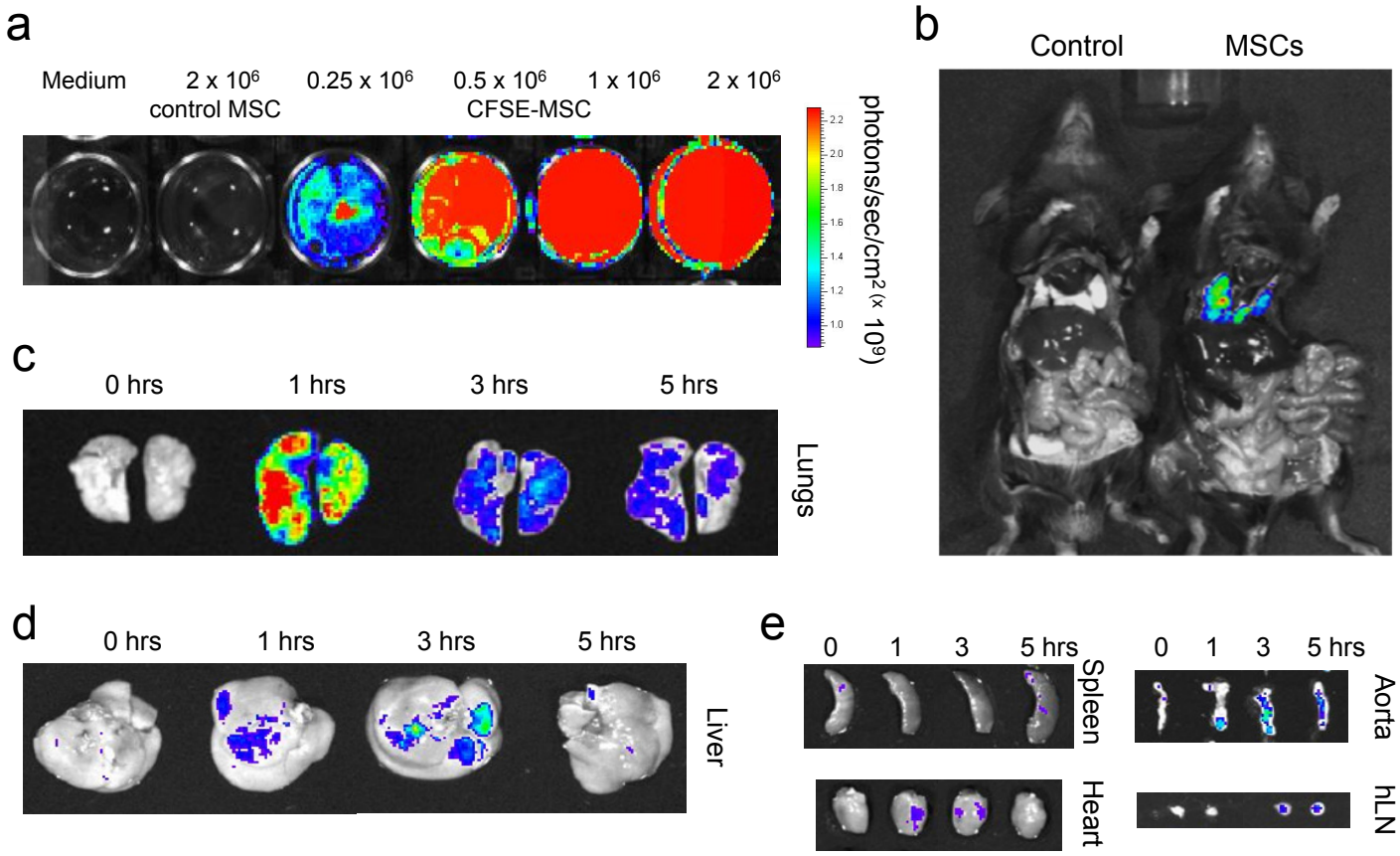

**Supplementary Figure 1. Phenotype of MSCs.** MSCs were generated from the bone marrow of male C57BL/6 mice and the expression of surface markers was analyzed by flow cytometry. Representative histograms are shown.



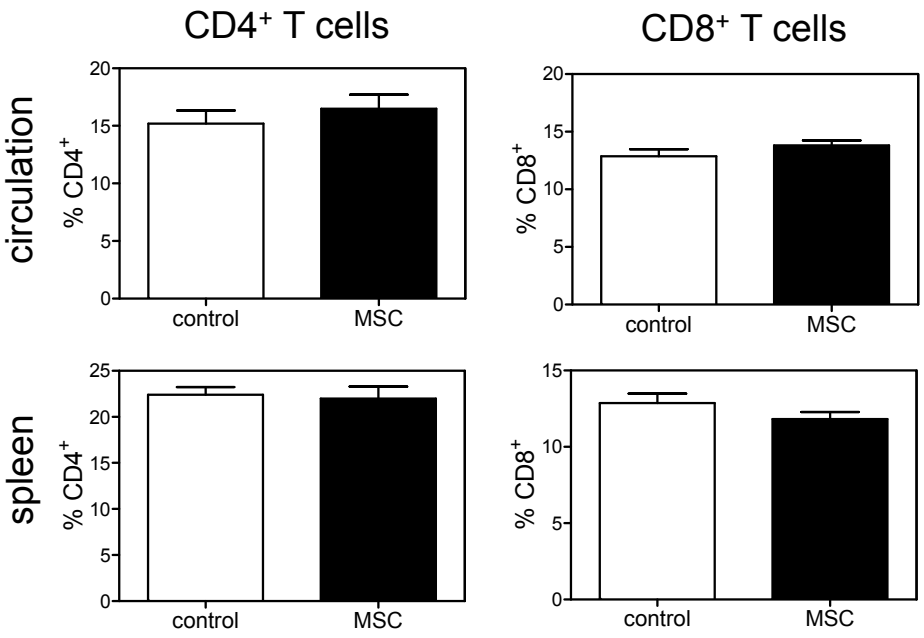

**Supplementary Figure 2. MSCs reduce T cell proliferation *in vitro.*** Total splenocytes and splenic CD4⁺ T cells, both obtained from LDLr KO mice, were co-cultured with indicated ratios of MSCs in the presence of αCD3/CD28 for 72 hours. Splenocyte and T cell numbers remained constant. a, Splenocytes showed reduced cytokine production in the presence of MSCs. b, Proliferation of CD4⁺ T cells was assessed by $^{3}$H-thymidine and by Ki-67 expression by flow cytometry. c, T cell subsets within CD4⁺ T cells were determined by flow cytometry. Cytokine production was determined by ELISA. All values are expressed as mean±SEM and representative of at least two independent experiments done in triplicate. * P<0.05, ** P<0.01, *** P<0.001. ND defines not determined.



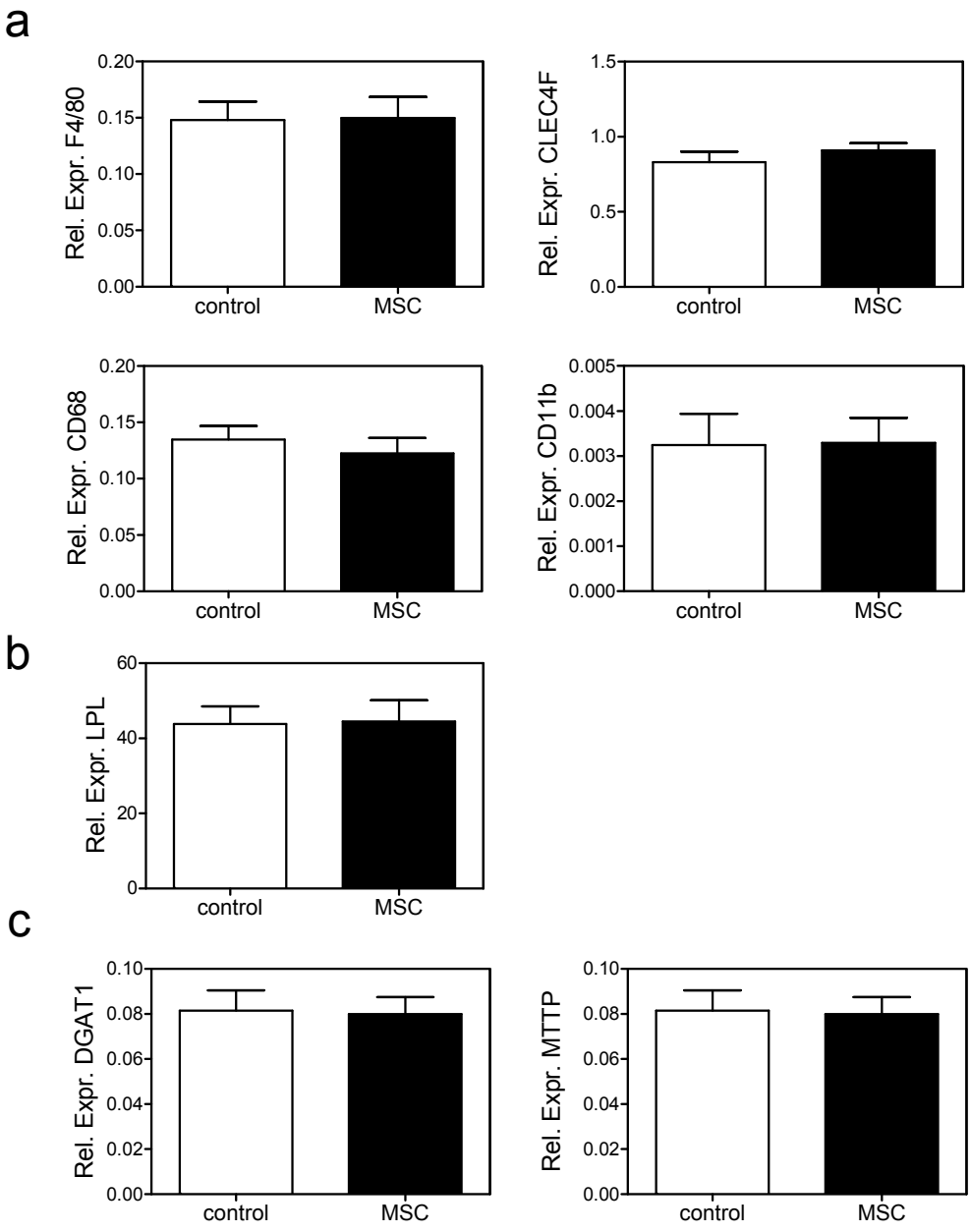

**Supplementary Figure 3. MSC supernatant does not affect T cell proliferation.** Splenocytes and $CD4^+$ T cells obtained from LDLr KO mice were cultured in the presence of MSC culture supernatant at the indicated amount in the presence of αCD3/28 for 72 hours. Proliferation was assessed by $^3$H-thymidine incorporation. Splenocyte and T cell numbers remained constant. All values are expressed as mean±SEM and representative of two independent experiments done in triplicate.



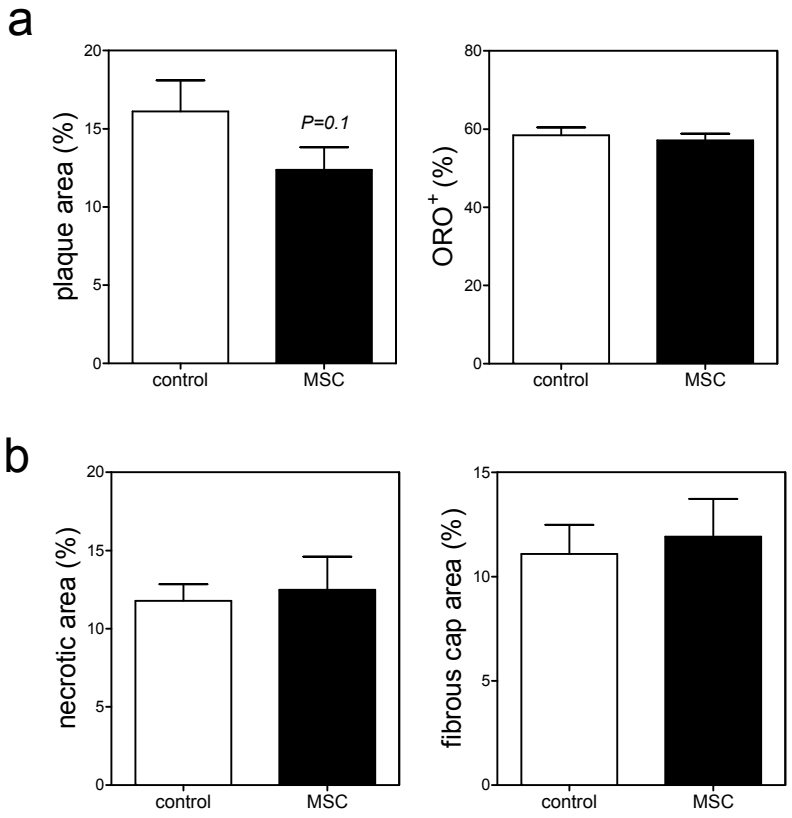

**Supplementary Figure 4. CFSE-labelled MSCs migrate preferentially to lung, liver, and the vasculature.** a, MSCs were labelled with 10μM CFSE. Signal intensity on IVIS correlates with amount of cultured $CFSE^+$ MSCs. Control MSCs indicates non-labelled MSCs. b, 15 min after *i.v.* injection of 1 x $10^6$ MSCs into LDLr KO, MSCs accumulate in the lung as determined by IVIS. c, MSC presence in the lung 1-5 hrs after injections into LDLr KO mice one week on WTD. d, liver and e, organ distribution of MSCs as determined by IVIS 1-5 hrs after injection of MSCs into LDLr KO mice one week on WTD.




**Supplementary Figure 5. MSC-treatment does not affect absolute CD4⁺ and CD8⁺ T cell numbers *in vivo*.** Male LDLr KO mice received three *i.v.* injections of either PBS (control) or 0.5 x $10^6$ MSCs (MSC) and were then fed a Western-type diet (WTD) for eight weeks. $CD4^+$ and $CD8^+$ T cells percentages were determined in the circulation and the spleen by flow cytometry. All values are expressed as mean±SEM and representative of six mice.




**Supplementary Figure 6. MSC treatment effect on mRNA expression in the liver.** a, Liver mRNA expression of Clec4f, F4/80, CD11b, and CD68 is shown. b, White adipose tissue mRNA expression of lipoprotein lipase (LPL) is shown. c, Liver mRNA expression of diglyceride acyltransferase 1 (DGAT1) and microsomal triglyceride transfer protein (MTTP), relative to the expression of three housekeeping genes (SDHA, HPRT and Rpl27). All values are expressed as mean±SEM and representative of all mice. *P<0.05, **P<0.01, ***P<0.001.




**Supplementary Figure 7. MSC treatment reduces lesion development but does not affect lesion composition.** a, The % plaque area was determined as total lesion area divided by total area of lumen and lesion. Lipid deposition was assessed by measuring Oil-red O positive area of lesions. b, necrotic area was determined as percentage acellular space of lesions and fibrous cap size as cap area divided by total lesion area.

| *Gene* | *Forward* | *Reverse* |
|---|---|---|
| **ACC1** | AGAATCTCCTGGTGACAATGCTTATT | GCTCTGTGAGGATATTTAGCAGCTC |
| **CD68** | TGCCTGACAAGGGACACTTCGGG | GCGGGTGATGCAGAAGGCGATG |
| **CLEC4F** | AGATCTGCAGGCGACCAAAGCC | TCTGCGCGATCAGCTGGAGAAC |
| **DGAT1** | GGTGCCCTGACAGAGCAGAT | CAGTAAGGCCACAGCTGCTG |
| **F4/80** | TGCAGGGGGCTTTCATCTTCCTCA | GGGAGCTAAGGTCAGTCTTCCTGGTG |
| **FASN** | GGCGGCACCTATGGCGAGG | CTCCAGCAGTGTGCGGTGGTC |
| **HPRT** | TACAGCCCCAAAATGGTTAAGG | AGTCAAGGGCATATCCAACAAC |
| **IL-6** | AGACAAAGCCAGAGTCCTTCAGAGA | GGAGAGCATTGGAAATTGGGGTAGG |
| **IL-10** | GGGTGAGAAGCTGAAGACCCTC | TGGCCTTGTAGACACCTTGGTC |
| **LPL** | CCCCTAGACAACGTCCACCTC | TGGGGGCTTCTGCATACTCAAA |
| **MTTP** | TCTCACAGTACCCGTTCTTGGT | GAGAGACATATCCCCTGCCTGT |
| **RPL27** | CGCCAAGCGATCCAAGATCAAGTCC | AGCTGGGTCCCTGAACACATCCTTG |
| **Scd1** | TACTACAAGCCCGGCCTCC | CAGCAGTACCAGGGCACCA |
| **SDHA** | TATATGGTGCAGAAGCTCGGAAGG | CCTGGATGGGCTTGGAGTAATCA |
| **SREBP-1C** | TCTGAGGAGGAGGGCAGGTTCCA | GGAAGGCAGGGGGCAGATAGCA |
| **SREBP-2** | TGAAGCTGGCCAATCAGAAAA | ACATCACTGTCCACCAGACTGC |
| **TNF-α** | GCCTCTTCTCATTCCTGCTTGTG | ATGATCTGAGTGTGAGGGTCTGG |

**Supplementary Table 1. Primer Pairs used for qPCR analysis.** The relative expression of genes was determined relative to the average expression of three housekeeping genes: hypoxanthine-guanine phosphoribosyltransferase (HPRT), succinate dehydrogenase complex, Subunit A (SDHA) and ribosomal protein L27 (Rpl27). Abbreviations: ACC1, Acetyl-CoA carboxylase 1; DGAT1, diglyceride acyltransferase 1; FASN, fatty acid synthase; LPL, lipoprotein lipase; MTTP, microsomal triglyceride transfer protein; Scd1; stearoyl-CoA desaturase-1; SREBP-1c, sterol regulatory element-binding protein 1c; SREBP-2, sterol regulatory element-binding protein 2.