Hematopoietic cell-specific SLC37A2 deficiency accelerates atherosclerosis in LDL receptor-deficient mice

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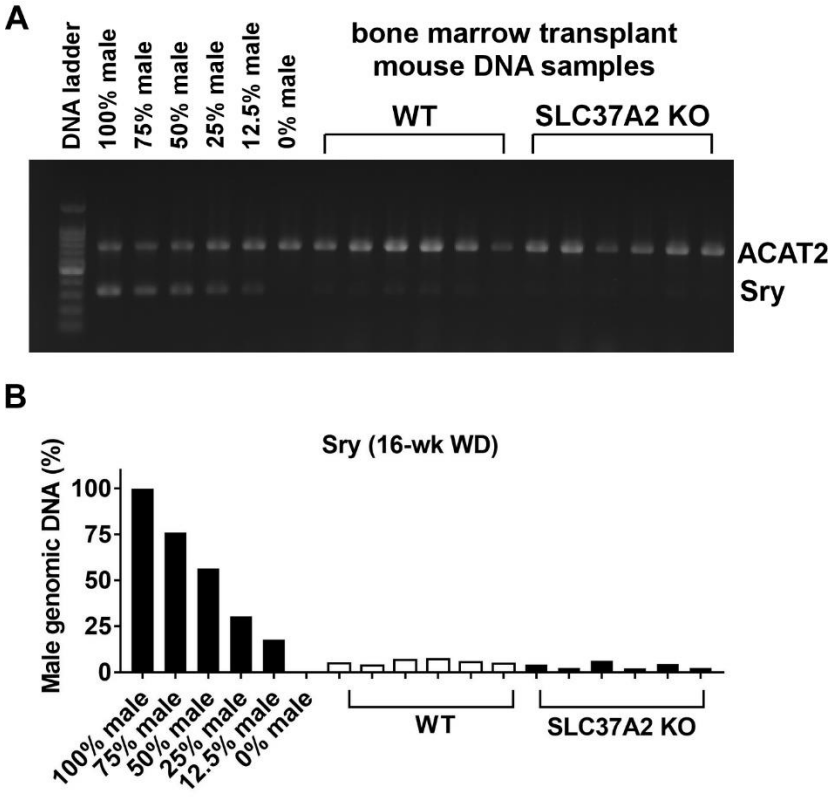

Figure S1. The efficiency of circulating hematopoietic cell replacement after bone marrow transplantation.

Sixteen wks after diet feeding, genomic DNA was isolated from whole blood. Fragments of the male Sry gene and ACAT2 (control) genes were amplified by PCR using genomic DNA as a template.

(**A**) A series of male and female genomic DNA mixtures were amplified to construct a standard curve based on the quantified ratio of Sry/ACAT2 band density and the actual percentage of the male/female genomic DNA mixture.

(**B**) Percentage of blood leukocyte female genomic DNA in male recipient mice was calculated based on the standard curve.

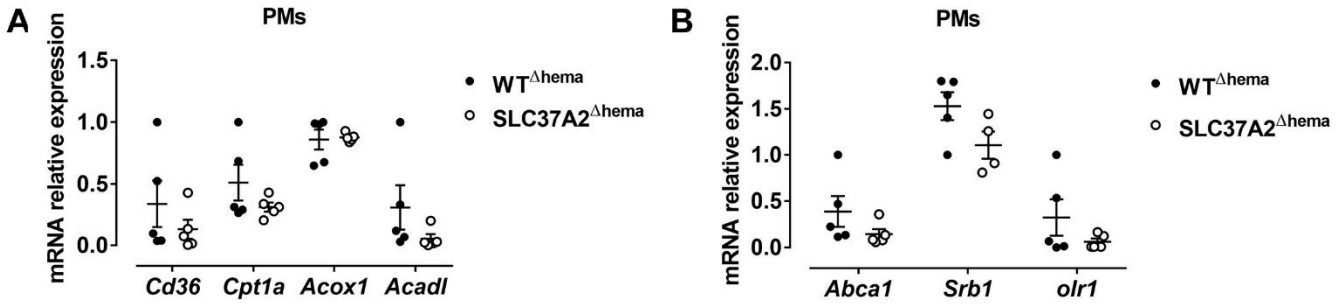

Figure S2. SLC37A2 deletion does not affect lipid metabolism-related gene expression in resident peritoneal macrophages from diet-fed mice.

(**A-B**) Relative transcript levels for genes encoding lipid metabolism in resident peritoneal macrophages. Each symbol represents an individual mouse. Data are expressed as mean ± SEM.

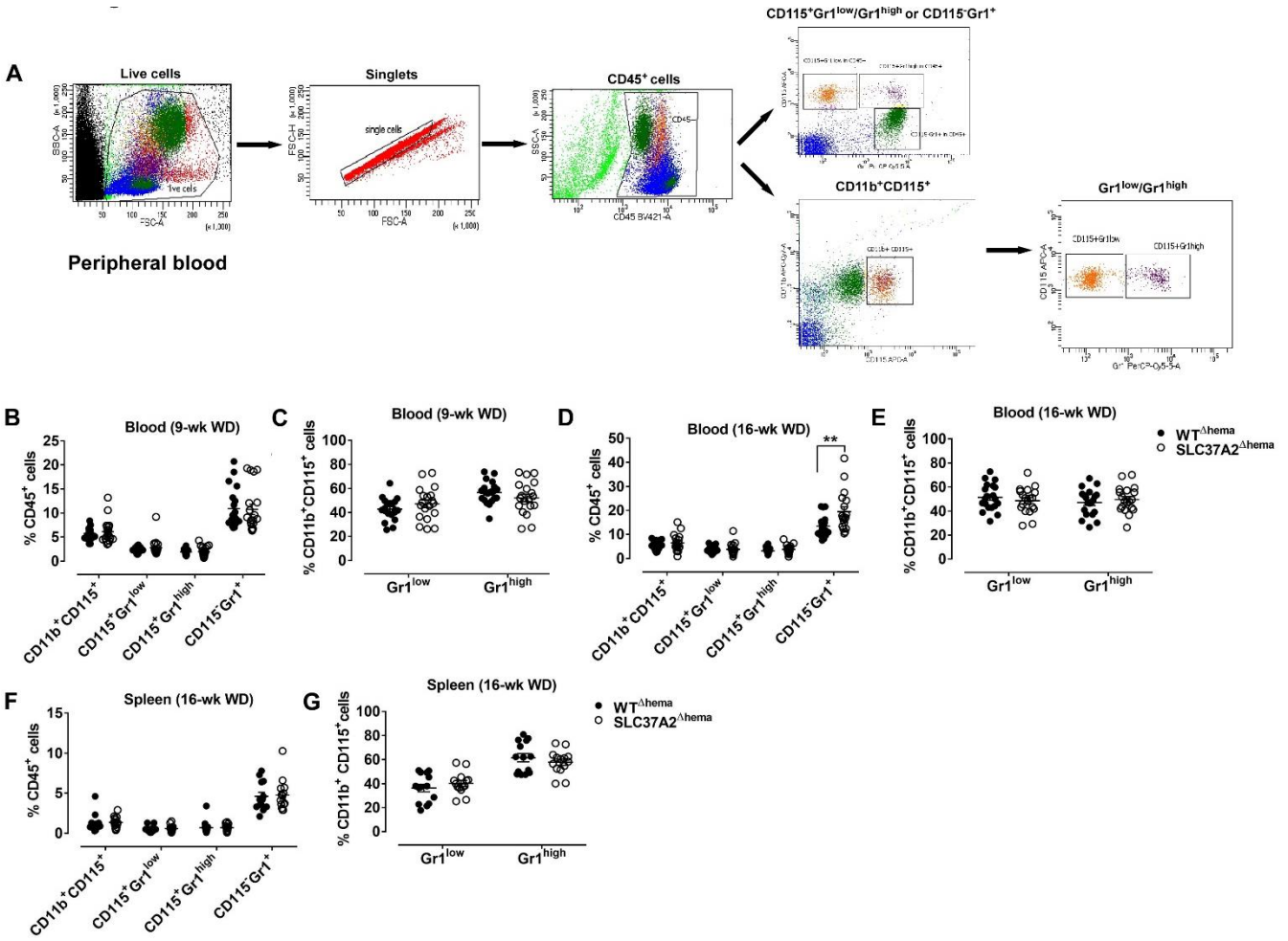

Figure S3. Hematopoietic SLC37A2 deletion has a minor effect on blood or spleen myeloid cell composition after 16-wks of diet feeding.

Irradiated Ldlr$^{-/-}$ mice transplanted with WT or SLC37A2KO bone marrow were fed a high-fat western diet for 16 wks. Blood cells and spleen cells were stained with CD11b-PE, CD115-APC, Gr1 (Ly6C/Ly6G)-PerCP-Cy5.5, and CD45-V450 and analyzed by flow cytometry.

(**A**) Gating strategies of the flow cytometry analysis for peripheral blood cells. Similar gating strategies were used to analyze the splenocytes.

(**B-C**) Percentages of monocytes (CD11b^{+}CD115^{+}), Gr1^{low} (CD11b^{+}CD115^{+}Gr1^{low}), Gr1^{high} (CD11b^{+}CD115^{+}Gr1^{high}), and neutrophils (CD11b^{+}CD115^{-}Ly6G^{+}) in circulating blood CD45^{+} cell (B) or blood monocytes (C) after 9-wk diet feeding.

(**D-E**) Percentages of monocytes (CD11b^{+}CD115^{+}), Gr1^{low} (CD11b^{+}CD115^{+}Gr1^{low}), Gr1^{high} (CD11b^{+}CD115^{+}Gr1^{high}), and neutrophils (CD11b^{+}CD115^{-}Ly6G^{+}) in circulating blood CD45^{+} cell (D) or blood monocytes (E) after 16-wk diet feeding.

(**F-G**) Percentages of monocytes (CD11b^{+}CD115^{+}), Gr1^{low} (CD11b^{+}CD115^{+}Gr1^{low}), Gr1^{high} (CD11b^{+}CD115^{+}Gr1^{high}), and neutrophils (CD11b^{+}CD115^{-}Ly6G^{+}) in splenic CD45^{+} cell (F) or monocytes (G) after 16-wk diet feeding.

Data are expressed as mean ± SEM. n = 12-16 mice per genotype. ** P<0.01, unpaired, two-tailed Student's t-test. Each symbol represents an individual mouse.

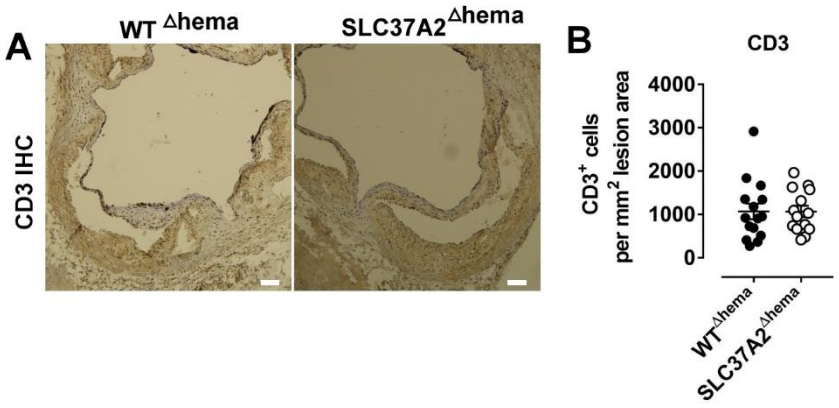

Figure S4. **Hematopoietic SLC37A2 deletion does not alter CD3⁺ cell (T cell) content in the aortic root intimal area.**

(**A-B**) Quantification of CD3⁺ cells (T cells) in the aortic root intimal area (number of CD3 positive cells in lesion). Scale bars = 100 μm.

Data are expressed as mean ± SEM. Each symbol represents an individual mouse. n = 12-16 mice per genotype. Unpaired, two-tailed Student's t-test.

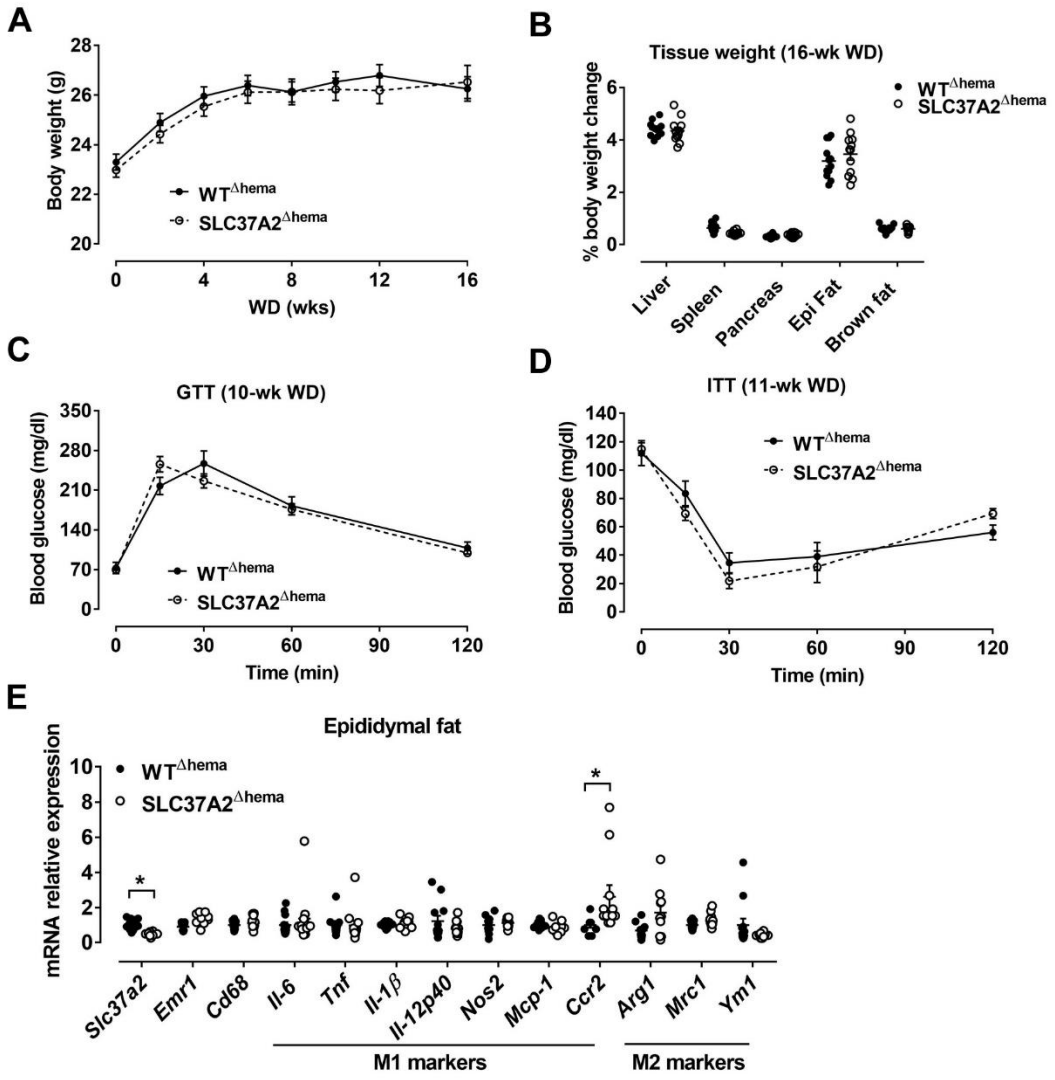

Figure S5. Hematopoietic SLC37A2 deletion has minimal impact on insulin resistance and adipose inflammation under pro-atherogenic conditions.

Irradiated Ldlr$^{-/-}$ mice receiving bone marrow from WT or SLC37A2KO mice were fed a high-fat western diet for 16 wks.

(**A**) Mouse body weight over the 16-wk diet feeding period.

(**B**) Mouse tissue weight after 16-wk diet feeding.

(**C**) GTTs were performed after 10-wk diet feeding.

(**D**) ITTs were performed after 11-wk diet feeding.

(**E**) Relative transcript levels of genes in epididymal fat from 16-wk diet-fed mice.

Data are expressed as mean ± SEM. n = 12-16 mice per genotype. * P<0.05, unpaired, two-tailed Student's t-test. Each symbol represents an individual mouse.

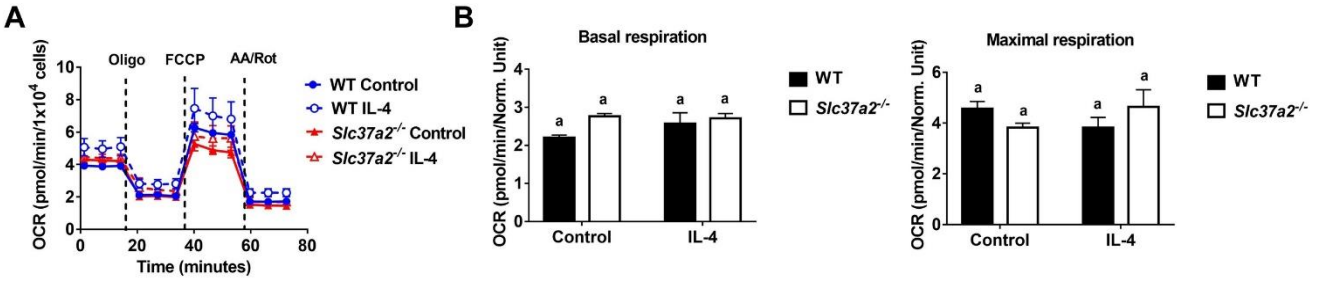

Figure S6. SLC37A2 deficiency does not affect macrophage mitochondrial respiration after 6 h of IL-4 treatment.

(A-B) Seahorse analysis of oxygen consumption rate (OCR) in WT and *Slc37a2*$^{-/-}$ BMDMs treated with or without 20 ng/ml IL-4 for 6 h.

Data are expressed as mean ± SEM. Bars with different letters denote significant among groups ($P < 0.05$); two-way ANOVA with post hoc Tukey's multiple comparisons test.

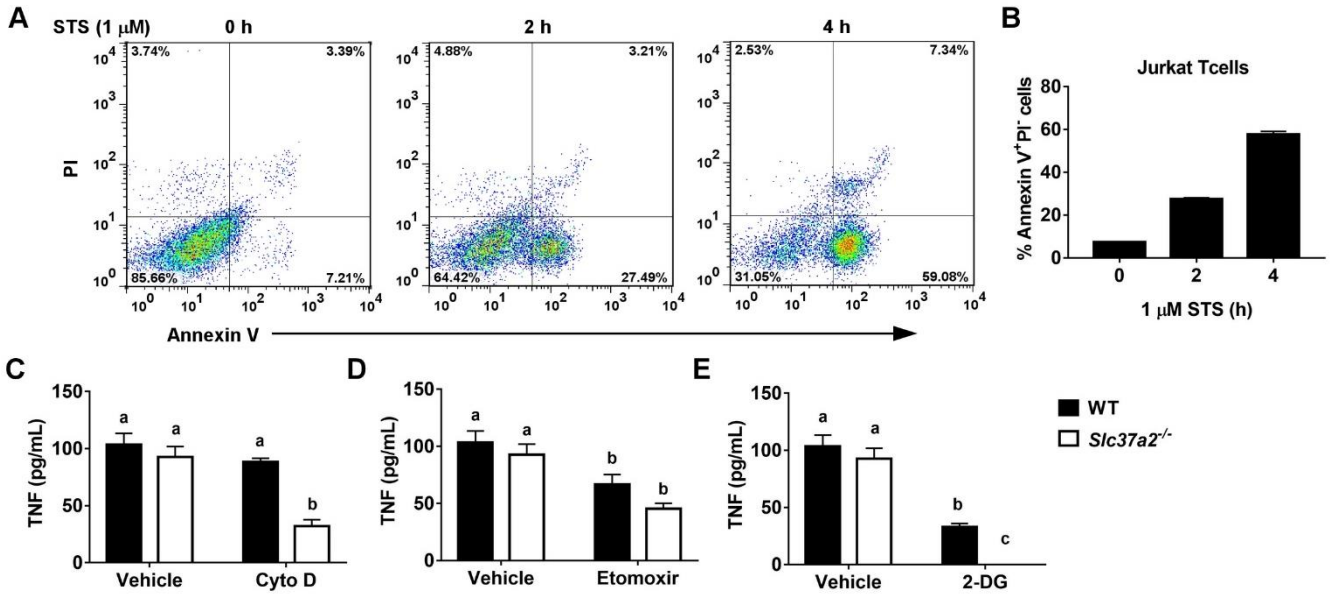

Figure S7. Flow cytometry analysis of apoptotic Jurkat T cells.

(**A-B**) Jurkat T cells were stimulated with 1 μM staurosporine for 0-4 h before stained with Annexin V-APC and PI. The percentage of early (Annexin V^+PI^-) and late apoptotic (Annexin V^+PI^+) cells was quantified by flow cytometry analysis. The numbers indicate percentages of the subpopulations (A). The percentage of early apoptotic cells during a 4-h period of staurosporine treatment (B).

(**C-E**) TNF secretion from WT and *Slc37a2*$^{-/-}$ BMDMs pretreated with phagocytosis inhibitor cytochalasin D (Cyto D, 10 μM), fatty acid oxidation inhibitor etomoxir (100 μM), and hexokinase inhibitor 2-deoxy-D-glucose (2-DG; 10 mM) for 30 min, followed by co-culture with ACs (macrophages: ACs= 1:5) for 4 h. Data are expressed as mean ± SEM. Bars with different letters differ significantly ($P < 0.05$); two-way ANOVA with post hoc Tukey's multiple comparisons test.

Table SI. Forward and reverse primers used in qPCR

Gene name	Forward primer (5'→3')	Reverse primer (5'→3')
Abca1	CGTTTCCGGGAAGTGTCCTA	GCTAGAGATGACAAGGAGGATGGA
Abcg1	GTT CAG GAG GCC ATG ATG GT	CCG TCT GCC TTC ATC CTT CTC
Acadl	TCTTTTCCTCGGAGCATGACA	GACCTCTCTACTCACTTCTCCAG
Acox1	AGATTGGTAGAAATTGCTGCAAAA	ACGCCACTTCCTTGCTCTTC
Arg1	AGCACTGAGGAAAGCTGGTC	CAGACCGTGGGTTCTTCACA
Ccr2	GTGTACATAGCAACAAGCCTCAAAG	CCCCCACATAGGGATCATGA
Cd36	GAGGAATCAGATGAGGATATGGGA	AAGCAGGCTGACTTGGTTGC
Cd68	CTTCCCACAGGCAGCACAG	AATGATGAGAGGCAGCAAGAGG
Cpt1a	CACCAACGGGCTCATCTTCTA	CAAAATGACCTAGCCTTCTATCGAA
Emr1	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
Gapdh	TGTGTCCGTCGTGGATCTGA	CCTGCTTCACCACCTTCTTGAT
Hmgcr	CTTGTGGAATGCCTTGTGATTG	AGCCGAAGCAGCACATGAT
Hmgcs	GCCGTGAACTGGGTCGAA	GCATATATAGCAATGTCTCCTGCAA
Il-1β	GTCACAAGAAACCATGGCACAT	GCCCATCAGAGGCAAGGA
Il-6	CTGCAAGAGACTTCCATCCAGTT	AGGGAAGGCCGTGGTTGT
Il-10	CAGAGCCACATGCTCCTAGA	TGTCCAGCTGGTCCTTTGTT
Il-12p40	AGACCCTGCCCATTGAACTG	GAAGCTGCTGCTGTTCTCATATT
Mcp-1	TTC CTCCACCACCATGCAG	CCAGCCGGCAACTGTGA
Mrc1	ACGAGCAGGTGCAGTTTACA	ACATCCCATAAGCCACCTGC
Olr1	CAAGATGAAGCCTGCGAATGA	ACCTGGCGTAATTGTGTCCAC
Pparc1a	AACCACACCCACAGGATCAGA	TCTTCGCTTTATTGCTCCATGA
Pparc1b	CGCTCCAGGAGACTGAATCCAG	CTTGACTACTGTCTGTGAGGC
Ppard	ACGCACCCTTTGTCATCCA	TTCCACACCAGGCCCTTCT
Pparg	CACAATGCCATCAGGTTTGG	GCTGGTCGATATCACTGGAGATC
Scd1	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAACC
Slc37a2	GCCTGCGGCAGAAGCAGTGG	AGCAGGGGTGGCCCATGTTG
Srb1	TCCCCATGAACTGTTCTGTGAA	TGCCCGATGCCCTTGA
Srebp1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Srebp2	GCGTTCTGGAGACCATGGA	ACAAAGTTGCTCTGAAAACAAATCA
Tnf	GGCTGCCCCGACTACGT	ACTTTCTCCTGGTATGAGATAGCAAAT
Ym1	AAGAACACTGAGCTAAAAACTCTCCT	GAGACCATGGCACTGAACG