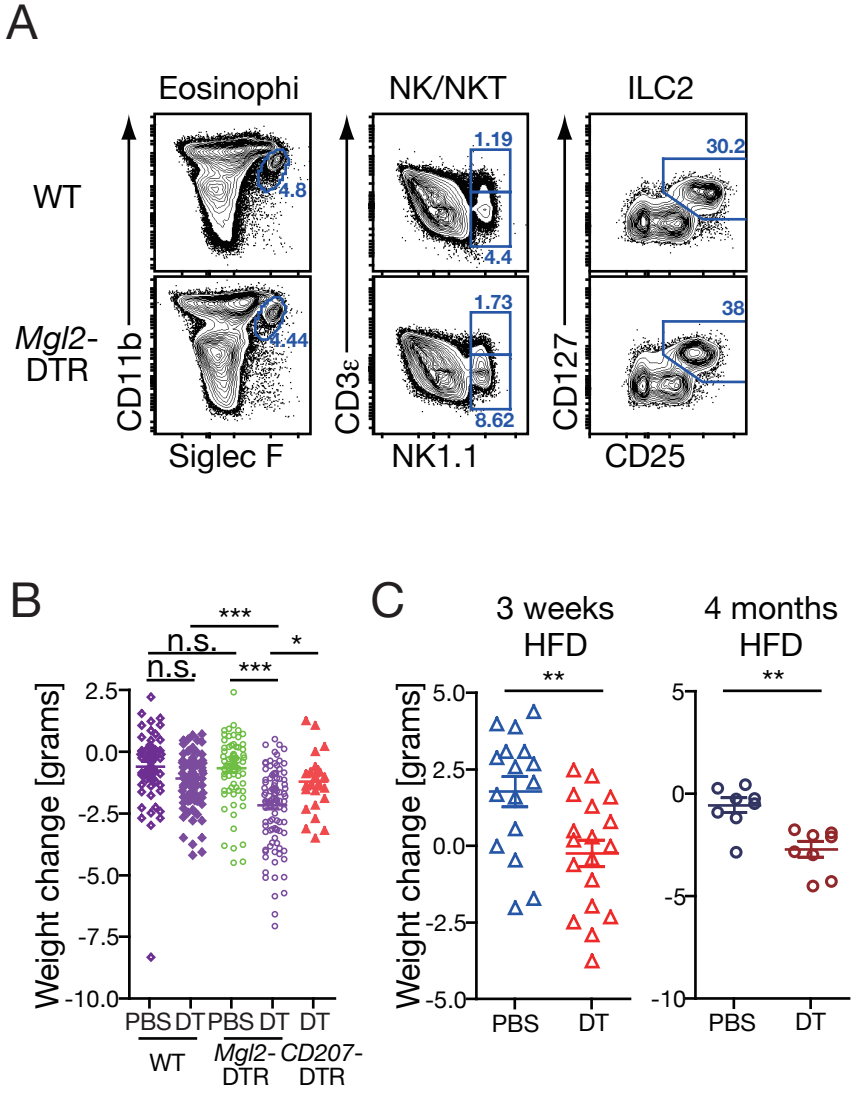
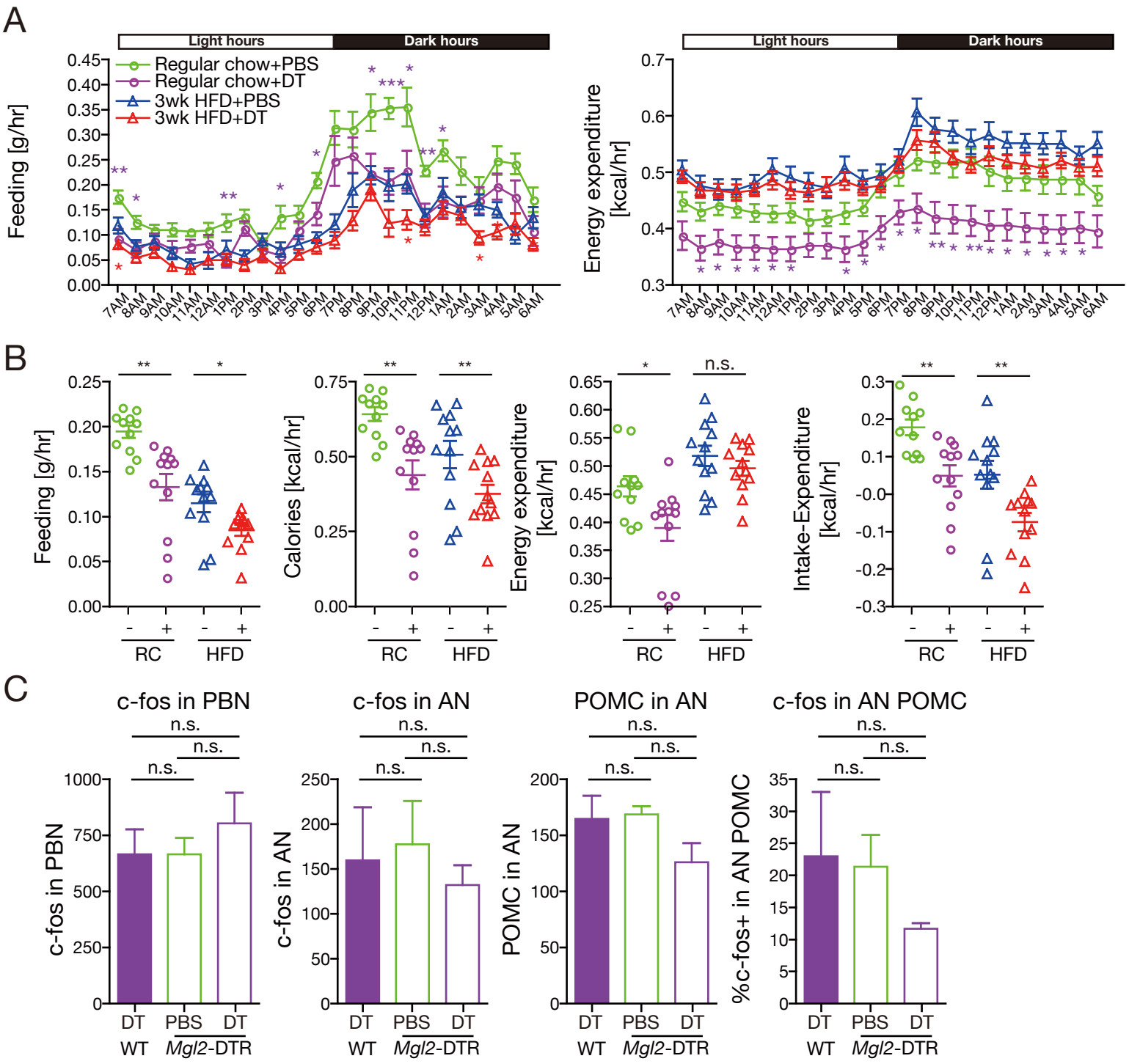
Supplementary Figure 1



Supplementary Figure 1 (related to Figure 2). Depletion of CD301b$^+$ MNPs results in weight loss. (**A**) Impact of DT treatment on non-MNP subsets in *Mgl2*-DTR mice. Regular chow-fed WT or *Mgl2*-DTR mice were treated with DT for 10 days as in **Figure 2A** and examined for the expression of indicated markers in the EWAT. Eosinophils (CD11b$^+$Siglec F$^+$) and NK or NKT cells (NK1.1$^+$CD3$\varepsilon^{-\text{ or }+}$) are gated on total CD45$^+$ cells and type 2 innate lymphoid cells (ILCS2, CD127$^+$CD25$^+$) are gated on CD45$^+$ CD3ε^- CD4$^-$ CD8α^- CD11b$^-$ CD19$^-$ CD49b$^-$ FcεRI$^-$ SiglecF$^-$ cells. (**B,C**) Weight loss in *Mgl2*-DTR mice after DT treatment is expressed in absolute weight.

Supplementary Figure 2

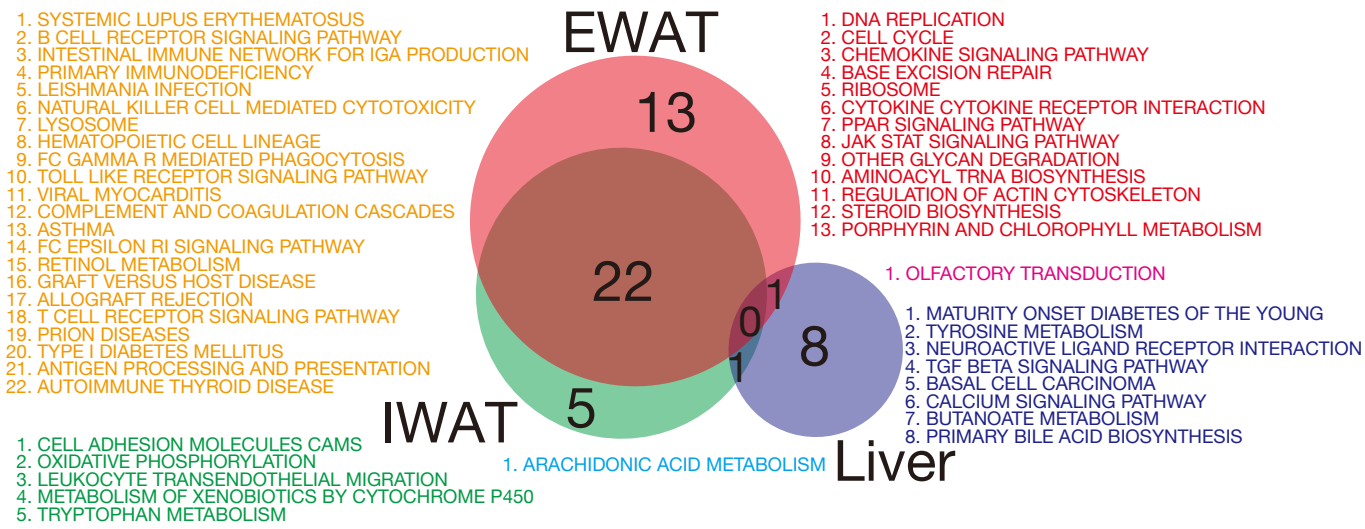


Supplementary Figure 2 (related to Figure 3). Depletion of CD301b⁺ MNPs results in a negative shift in the energy balance.

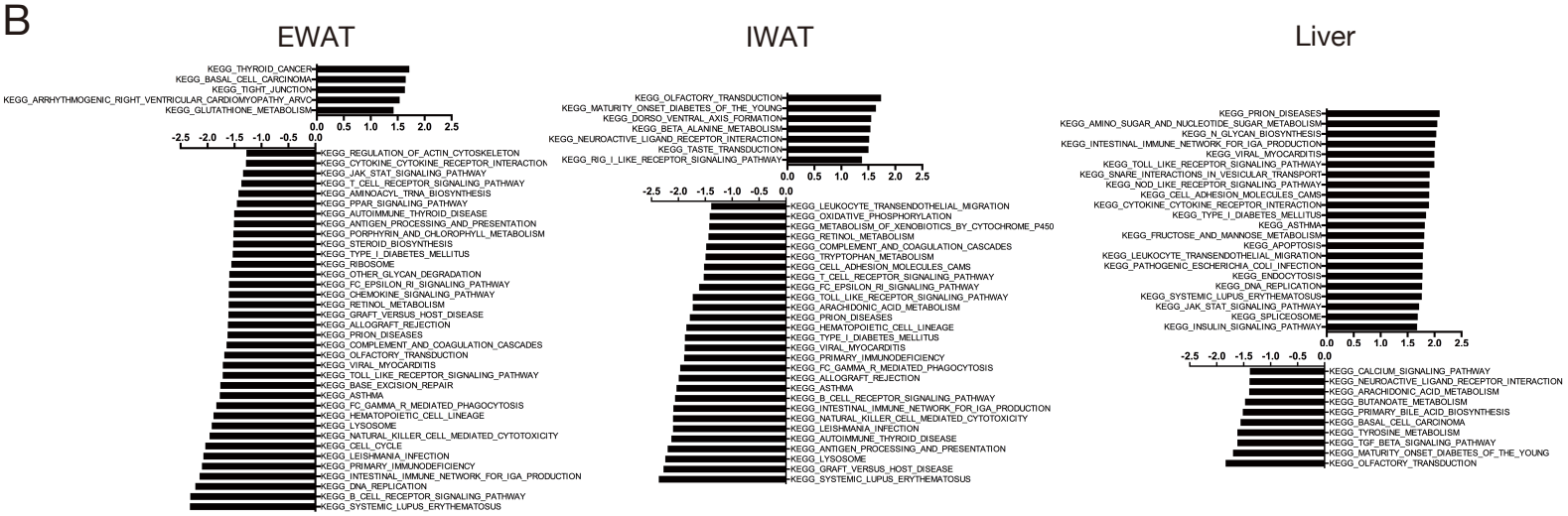
(**A,B**) Data shown in **Figure 3 A** and **B** are presented as per mouse values. (**C**) After 10 days of DT treatment, brains were fixed by perfusion and stained for c-fos and proopiomelanocortin (POMC). Positive signals in parabrachial nucleus (PBN) and arcuate nucleus (AN) were counted in five sections per mouse, three mice per group.

Supplementary Figure 3

A



B



Supplementary Figure 3 (related to Figure 6). Depletion of CD301b⁺ MNPs affects distinct pathways in the WAT and liver.

Gene set enrichment analysis of the microarray data shown in **Figure 6**. Differentially regulated KEGG pathways with nominal p<0.05 are shown.

Supplemental Experimental Procedures

Mice and treatments

Colonies of WT C57BL/6 (WT), *Mgl2*-DTR (*Mgl2*$^{+/DTReGFP}$) and homozygotic *Mgl2*$^{DTReGFP/DTReGFP}$ mice were maintained in our specific pathogen-free facility at room temperature (20-22ºC). *CD207*-DTR mice were a gift from Daniel Kaplan and maintained in our colony. Transgene-bearing *CD207*-DTR mice were screened as previously described (Bobr et al., 2010). YARG mice were a gift from Ruslan Medzhitov. *Adipoq*$^{-/-}$ mice were purchased from the Jackson Laboratory and crossed with *Mgl2*$^{DTReGFP/DTReGFP}$ to make *Mgl2*$^{+/DTReGFP}$*;Adipoq*$^{-/-}$ mice. Unless otherwise stated, 3-6 month-old males were used for experiments. Where indicated, mice were fed with HFD (60% kcal% fat, D12492, Research Diets) for indicated period of time. For *in vivo* cell depletion analyses, DT (0.5 μg/mouse/dose, List Biological Laboratories) was given intraperitoneally every three days for four times, then mice were sacrificed on day 10. In HFD-fed animals, DT was given in the last 10 days of the indicated HFD feeding period. For chemical sympathectomy, WT mice were injected intraperitoneally twice with 3.5 mg/mouse/dose 6-hydroxydopamine (~100 μg/g body weight, MP Biomedicals) dissolved in 0.07% (w/v) ascorbic acid (Sigma) in PBS on 1 and 3 days prior to the first DT treatment, as previously described (Riol-Blanco et al., 2014). For reconstitution of RELMα, 10 μg of bacteria-expressed RELMα (Peprotech) was injected intraperitoneally every three days for four times as previously described (Osborne et al., 2013), at the same time with the DT treatments. In general, animals with different treatments were co-housed in the same cages to avoid cage-to-cage variation. For indirect calorimetry, mice were individually housed during the 10-day DT treatment

period and their metabolic activity was monitored in metabolic cages in the last five days. All animal protocols were approved by the Institutional Animal Care and Use Committee at Yale University.

Cell preparation and flow cytometry

For adipose-tissue leukocytes, the fat pad was minced and digested with 2 mg/ml collagenase D (Roche), then the stromal vascular fraction was collected by centrifuging at 500 x g for 5 minutes. For colonic MNPs, the colonic epithelium was removed by 5 mM EDTA, then the remaining tissue was minced and digested with collagenase D. Peritoneal leukocytes were collected from naïve WT mice by flushing the peritoneal cavity with PBS. Blood leukocytes were collected by separating the blood with Histopaque-1077 (Sigma). For isolating cells from the adipose tissue, liver, skeletal muscle and pancreas, mice were briefly perfused with PBS, then the organs were removed, minced and digested with 5 mg/ml collagenase D and 2 mg/ml DNase I (Roche). For hepatic leukocytes, the resulting cell suspensions were then further separated by Histopaque-1077 by collecting the interphase after centrifuging at 500 x g for 20 minutes. Cells were stained with monoclonal antibodies against CD11b (clone M1/70), CD11c (N418), F4/80 (BM8), MHCII (M5/114.15.2), CD206 (C068C2), and CD301b (11A10-B7) or rat IgG2a isotype control (RTK2758). All monoclonal antibodies were purchased from Biolegend except anti-CD301b, which was developed and prepared in-house (Kumamoto et al., 2013). For intracellular RELMα staining, cells pre-stained for MNP markers were fixed with Fixation/Permiabilization Reagent (eBioscience) and stained with biotinylated rabbit anti-mouse RELMα (Peprotech),

followed by detection with streptaviding-PE-Dazzle 594 (BioLegend).

Gene expression analyses

Harvested tissues were immediately minced and lysed in Trizol (Invitrogen) and stored frozen at -80ºC. Total RNA was isolated with chloroform and isopropanol , then cleaned up with RNeasy Mini Kit (Qiagen). RNA isolated from epididymal and inguinal WAT and the liver was analyzed with Affymetrix Mouse Gene 2.0ST Microarray according to manufacturer's protocol. For real-time PCR, cDNA was synthesized with iScript cDNA synthesis kit (Bio-Rad). Genes were amplified with iTaq Universal SYBR Green Supermix (Bio-Rad) with the primers listed in Supplementary Information and their relative amount to *Hprt* was calculated by quantitative real-time PCR.

ELISA and metabolite measurements

Sera were stored frozen at -80ºC upon harvest. Serum leptin (Mouse Leptin (OB) ELISA Kit, Sigma), adiponectin (Adiponectin (mouse) ELISA Kit, Adipogen), free and total cholesterol (Total Cholesterol and Cholesteryl Ester Colorimetric Assay Kit II, BioVision), free and total T3 (fT3 and total T3 ELISA Kit, Alpha Diagnostic) and corticosterone (Corticosterone ELISA Kit, Abnova) were measured according to manufacturer's protocol. For quantifying serum RELMα, ELISA plates were coated with 50 µl/well, 2 µg/ml rabbit anti-murine RELMα and serum RELMα was detected by 50 µl/well, 2 µg/ml biotinylated rabbit anti-murine RELMα using recombinant RELMα (all reagents are from Peprotech) as quantification standard.

Glucose tolerance tests and hyperinsulinemic euglycemic clamps

Experiments evaluating glucose homeostasis were performed according to recommendations by the NIH-funded Mouse Metabolic Phenotyping Center (MMPC) consortium (Ayala et al., 2010). Glucose tolerance tests were performed after an overnight fast. Blood was collected by tail bleed for measures of plasma glucose and insulin at set time points following an i.p. challenge of 1 mg/g body weight dextrose. Glucose was measured using a YSI Glucose Analyzer or a Breeze2 Glucometer (Bayer) and insulin was measured by radioimmunoassay (Linco). Hyperinsulinemic euglycemic clamps were performed as previously described (Jurczak et al., 2012). After surgical implantation of an indwelling catheter in the right jugular vein, mice were allowed to recover 1 week prior to clamp experiments. Following an overnight 14h fast, mice were infused with 3-^{3}H-glucose at a rate of 0.05 μCi/min for 120 min to determine basal glucose turnover. Next, a primed infusion of insulin and 3-^{3}H-glucose was administered at 7.14 mU·kg^{-1}·min^{-1} and 0.24 μCi/min, respectively, for 4 min, after which rates were reduced to 3 mU·kg^{-1}·min^{-1} insulin and 0.1 μCi/min 3-^{3}H-glucose for the remainder of the experiment. Blood was collected via tail massage for monitoring plasma glucose, insulin and tracer at set time points during the 140 min infusion, and a variable infusion of 20% dextrose was given to maintain euglycemia. Glucose turnover was calculated as the ratio of the 3-^{3}H-glucose infusion rate to the specific activity of plasma glucose at the end of the basal infusion and during the last 40 min of the hyperinsulinemic-euglycemic clamp study. Hepatic glucose production represents the difference between the glucose infusion rate and the rate of glucose appearance. A 10 μCi bolus injection of ^{14}C-2-deoxyglucose was given at 90 min to determine tissue-specific glucose uptake,

which was calculated from the area under the curve of ^{14}C-2-deoxyglucose detected in plasma and the tissue content of ^{14}C-2-deoxyglucose-6-phosphate. Following collection of the final blood sample, mice were anesthetized with an intravenous injection of 150 mg/kg pentobarbital and tissues were harvested and froze with aluminum forceps in liquid nitrogen. All tissues were stored at -80^{o} C until later use.

c-Fos staining in the brain

Under deep anesthesia, mice were perfused with a fixative containing 4% paraformaldehyde, 15% picric acid, 0.1% glutaraldehyde in 0.1 M phosphate buffer. Fifty micron sections were cut through the ARN and PBN of the brain, and every third section was used for immunostaining. For staining ARN, sections were stained with goat anti-c-Fos (Santa Cruz) and, where applicable, with rabbit anti-POMC (Phoenix Pharmaceuticals), followed by visualization with Alexa fluor 594 donkey anti-rabbit and Alexa fluor 488 donkey anti-goat (Life Technologies) in a Zeiss Axioplan 2 imaging system. For staining PBN, sections were incubated with goat anti-c-Fos, then with biotinylated donkey anti-goat IgG (Vector Labs). Specific antibody binding was detected by avidin-coujugated peroxidase (ABC, Vector Labs) with diaminobenzidine.

PCR primers

Gene	Forward primer	Reverse primer
Adipor1	CTCATCTACCTCTCCATCGTCTGT	TACAACACCACTCAAGCCAAGTC
Ces2c	AATGATGCTGTCTTCCTTCCCT	CACACCATCACAGGCAGGTTA
Ces3b	CAATTCCCCACTGTGCCCTAC	GGCACTTCCATAATGGCCAAG
Cyp17a1	GAAAGAATTCTCTGGTCGGCCC	CCTGACATATCATCTTCTCCAGTTTC
Cyp8b1	CGGAGATAAGGTGGCTCTCTTC	TGTAGTGGTGGATCTTCTTGCC
Dio1	CTGGCAGAGACTGGAAGACAG	GGCCTGCTGCCTTGAATGAAAT
Elovl3	CATCCATGAATTTCTCACGCGG	AGCTTACCCAGTACTCCTCCA
G6pdx	GGGAAGAGTTGTACCAGGGTGA	ACAGCCACCAGATGGTAGGATAGAT
Hprt	GTTGGATACAGGCCAGACTTTGTTG	GAGGGTAGGCTGGCCTATTGGCT
Hsd11b1	TGCGAAGAGTCATGGAGGTC	GGAGCAATCATAGGCTGGGT
Hsd3b4	GAAGAGGAATTGTCCAAGCTGC	CTAGGATGGTCTGTCTGGAAGC
Hsd3b5	GCTCTTGGAAACAAAAGGAACACTT	ATCCTCTGGCCAAGAAACCCT
Mgl2	GACTGAGTTCTCGCCTCTGG	TGACAAACGCAAGGCTTCTG
Mrc1	TCTCTGTCATCCCTGTCTCTGTT	GAGATGGCACTTAGAGCGTCC
Retnla	TGCCAATCCAGCTAACTATCCC	ATCTCCACTCTGGATCTCCCAA
Scd1	GAGTACGTCTGGAGGAACAT	GCCCAGAGCGCTGGTCATGT
Serpina7	ACTGCAGAAAGGATGGGTTGA	TCAGCACTTTCAGCAAAGGCA
Slco1a1	AAGAAAGGCCCTGAGTGTGAC	TGCCGGAATGCCAGCTAATAGTC
Srebf1	ACAGCGGTTTTGAACGACAT	AGCATAGGGGGCGTCAAAC
Thrsp	CAGGAGGTGACGCGGAAATAC	TTCTCTCGTGTAAAGCGATCTTCAG
Ucp1	AGCGTACCAAGCTGTGCGATGT	ACATGATGACGTTCCAGGACCCGA