

Supplemental File 1: Additional Information for Research Design and Methods

Animals

Angptl4^{+/+} and *Angptl4*^{-/-} mixed C57BL/6J:129 SV background male mice (1) were cohoused as in a temperature-controlled room of approximately 22°C with 30-70% humidity in ventilated cages with a 12 hour light and dark cycle. Cages include Sanichip bedding along with a cotton Nestlet and a 4 g pick of crinkled paper. Mice were cohoused and were fed ad libitum a diet of PicoLab Rodent diet 5053 which contains 20% protein, crude fat 4.5%, and Fiber 6.0%. For experiments, randomly assigned male mice 8-15 weeks old were used. All experiments were approved by the University of California, Berkeley (AUP-2014-07-6617)

AAV

S1PR1 shRNA sequences:

(CCGGCCATGTAAACTGGGTCAAGTTCTCGAGAACTTGACCCAGTTTACATGGTTTTT

), S1PR2 shRNA sequences:

(CCGGGCCATCGTGGTGGAGAATCTTCTCGAGAAGATTCTCCACCACGATGGCTTTT),

S1PR3 shRNA sequences:

(CCGGTTGGGATGTGCTGGCTAATTGCTCGAGCAATTAGCCAGCACATCCCAATTTTT

G), *RORγ* shRNA sequences:

(CACCGGAGCAGACACACTTACATACCTCGAGGTATGTAAGTGTGTCTGCTCCTTTTT

)

RNA-seq

Total RNA underwent sample QC using the Agilent 2100 Bio analyzer for total RNA sample QC: RNA concentration, RIN value, 28S/18S and fragment length distribution. BGI's SOAPnuke was used to filter reads and clean reads were stored in FASTQ format (version v1.5.2, parameters: -n.0.001 -l 20 -q 0.4 -A 0.25). HISAT2 was used for mapping (version: v2.0.4, parameters: -p 8 -phred33 -sensitive -I 1 -X 1000). Clean reads were mapped using Bowtie 2 and gene expression level was calculated (Bowtie2 version: v2.2.5, parameters: -q -phred33 -sensitive -dpad 0 -gbar 99999999 -mp 1, 1 -np 1 -score-min L, 0, -0,1 -p 16 -k 200) (RSEM version: v1.2.12, parameters: default). Gene expression cluster analysis was displayed with javaTreeview using cluster software (version: v3.0, parameters: -g 7 -e 7 -m a). DEGs were detected with DEseq2 PossionDIs (DEseq2 parameters: fold change ≥ 2.00 and adjusted p value ≤ 0.05) (PossionDis parameters: fold change ≥ 2.00 and FDR ≤ 0.001). Gene ontology analysis of DEG and pathway analysis of DEG were performed. FASTQ files were uploaded to NCBI's GEO.

ELISA

16hr fasted plasma was used for insulin ELISA (Crystal Chem Inc, 90080). For pAkt and Akt levels, PBS or 10U/kg insulin (Sigma, I0516-5ml) were injected 10 minutes before euthanasia. 50mg of tissues were homogenized in cell lysis buffer and BCA assay was performed (Thermo Fisher, 23225) before being measured using the Akt (total) ELISA kit (Invitrogen, 85-86046-11).

S1P ELISA (ABclonal RK00714) was used to measure plasma and liver S1P levels. 50µl plasma was used directly in the kit while 100mg liver was rinsed with cold PBS, homogenized in 500µl lysis buffer, spun 10,000g, 5 min 4°C, and 50µl of the supernatant was used for analysis.

Western Blot

The following antibodies were used: GAPDH (Proteintech, 10494-1-AP), beta-actin (Sigma, A2066-100uL), S1PR2 (Abcam, ab235919), S1PR1 (Proteintech, 55133-1-AP), S1PR2 (Thermo, PA5-23208), S1PR3 (Invitrogen, PA5-23225), ROR γ (R&D systems, MAB6109), ROR α (Proteintech 10616-1-AP), Akt (p and total) (Cell Signaling, 9271S, 9272S), H3 (Abcam ab1791), anti-mouse or anti-rabbit 800 (Li-Cor, 926-32210/926-32211).

Cell Culture and Transfection

H4IIE Cells were transfected using Lipofectamine 3000 with the listed amounts of human ROR γ (Novus, NBP2-25278) and pCMV (control) along with 300ng human glucocorticoid receptor, 40ng of Renilla, and 500ng of the rat *Pck1* reporter overnight. The rat *Pck1* firefly luciferase was generated by subcloning the rat *Pck1* promoter from -2100 to +69 (relative to transcription start site) from pPL1CAT (2) into the polylinker of the plasmid pGL3-Basic (Promega). Cells were then washed with PBS and serum starved for 2 hours before being treated with 500nM dexamethasone (Sigma-Aldrich) or with 500nM dexamethasone and LYC-55716 (MedChemExpress, HY-104037). After 24 hours, cells were harvested in Passive Lysis Buffer (Promega) and luciferase activity was measured following manufacturer instructions from the

Dual-Luciferase Reporter Assay System (Promega, E1980). Luciferase activity was normalized by protein concentration of cell lysate using the Pierce BCA Protein Assay (ThermoFisher Scientific, 23225).

Reference

1. Chen TC, Lee RA, Tsai SL, Kanamaluru D, Gray NE, Yiv N, Cheang RT, Tan JH, Lee JY, Fitch MD, Hellerstein MK, Wang JC: An ANGPTL4-ceramide-protein kinase C ζ axis mediates chronic glucocorticoid exposure-induced hepatic steatosis and hypertriglyceridemia in mice. *J Biol Chem* 2019;294:9213-9224
2. Magnuson MA, Quinn PG, Granner DK: Multihormonal regulation of phosphoenolpyruvate carboxykinase-chloramphenicol acetyltransferase fusion genes. Insulin's effects oppose those of cAMP and dexamethasone. *J Biol Chem* 1987;262:14917-14920