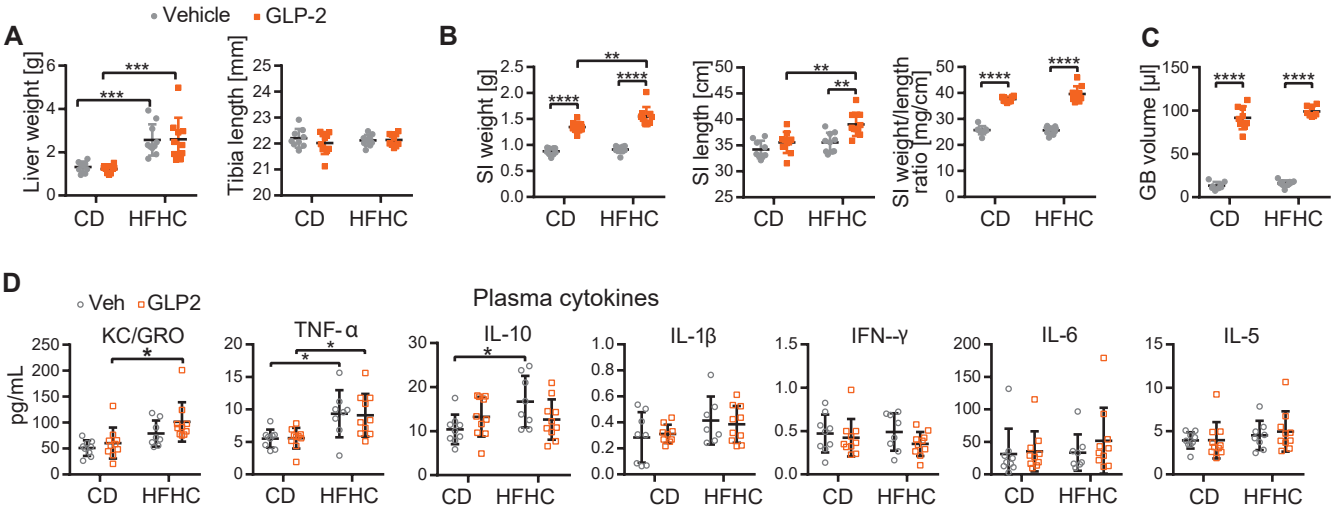
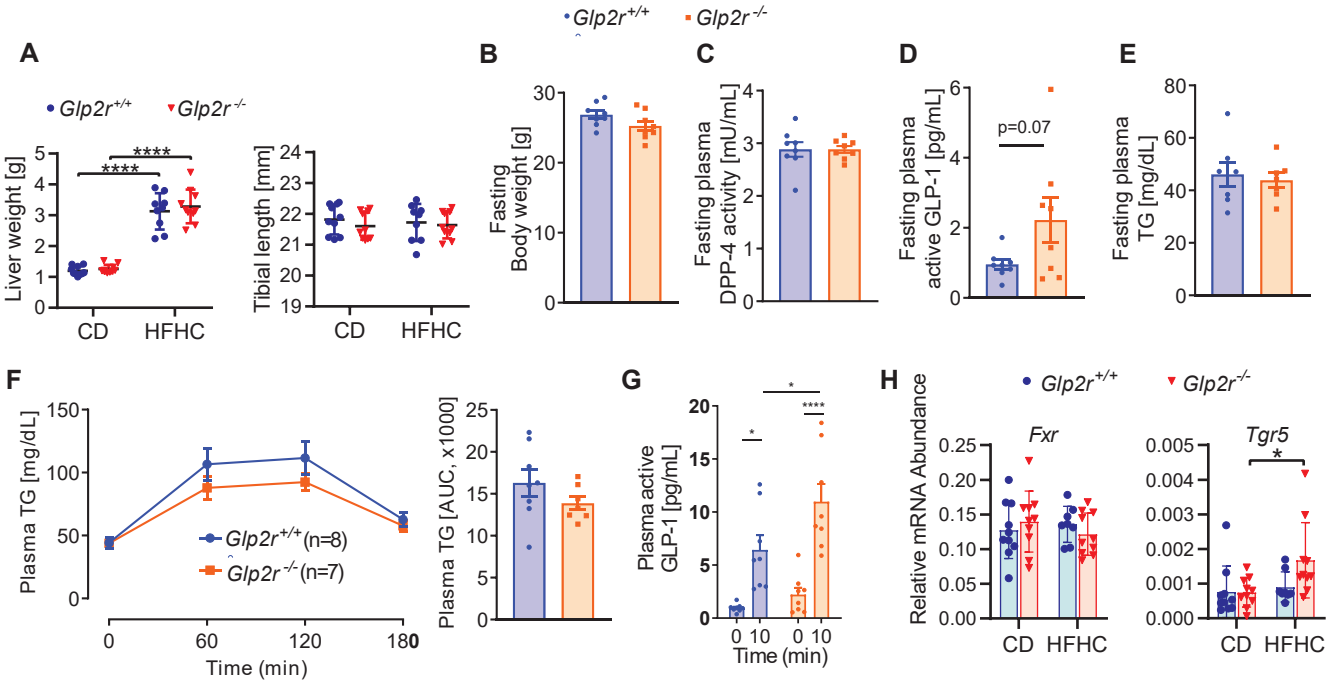
Supplemental Figures



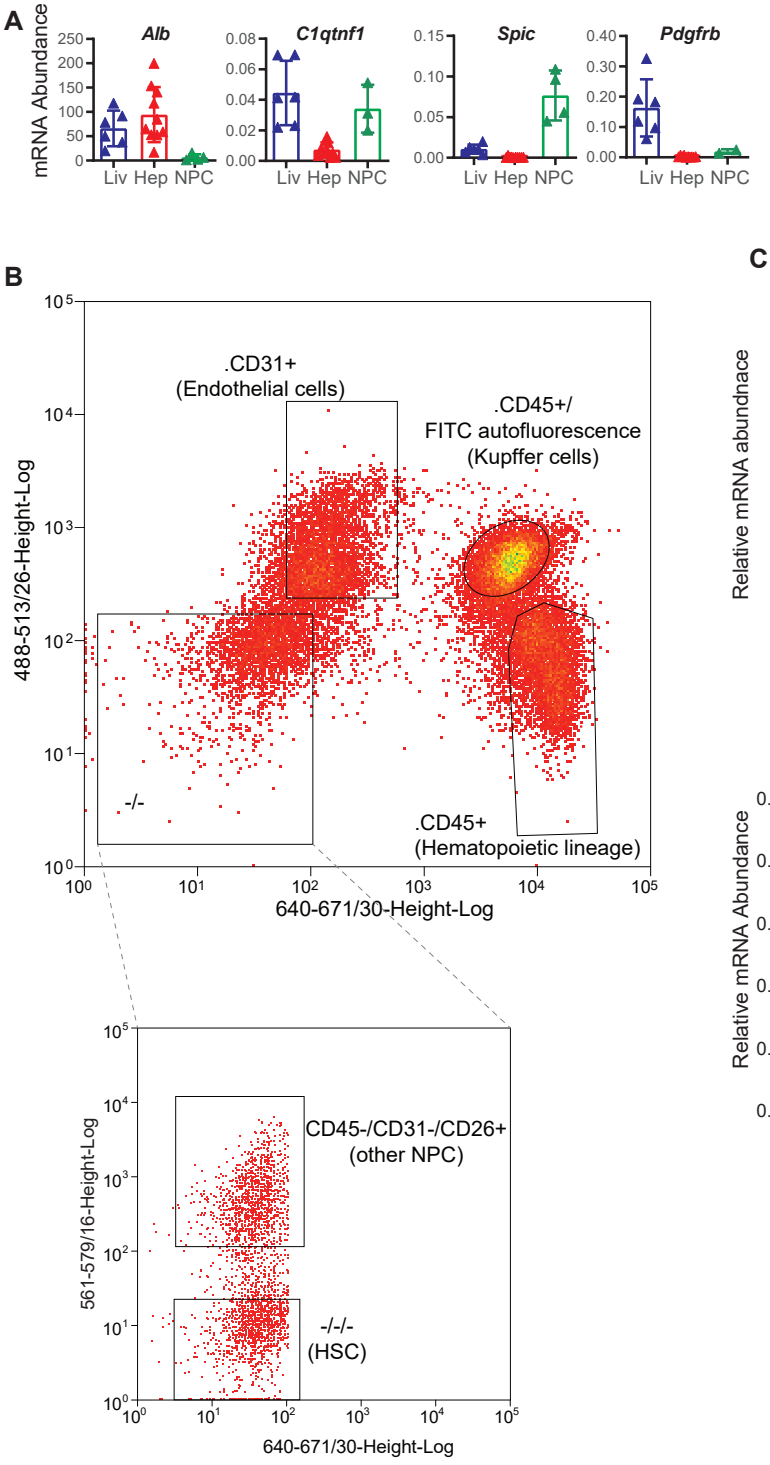
Supplemental Figure S1 Endpoints in mice on HFHC or CD diet treated with GLP-2 or vehicle.

(A) Liver weight (left) and tibia length (right). Tibia length was used for normalization of all measures reported in panels S1A and S1B. (B) Indicators of GLP-2 treatment efficacy: small intestine (SI) weight, length and weight/length ratio. (C) Gallbladder (GB) volume determined by image analysis of GBs that were ligated and resected at termination. (D) Concentrations of inflammatory cytokines in plasma. For (A-D), mice were treated daily with GLP-2 or vehicle for 11 days, with the last dose administered one hour prior to termination, n=9-10 per group. for all panels. Data are presented as the means ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using two-way ANOVA with Tukey correction for multiple comparisons.



Supplemental Figure S2 Metabolic phenotypes in CD- vs. HFHC-fed mice.

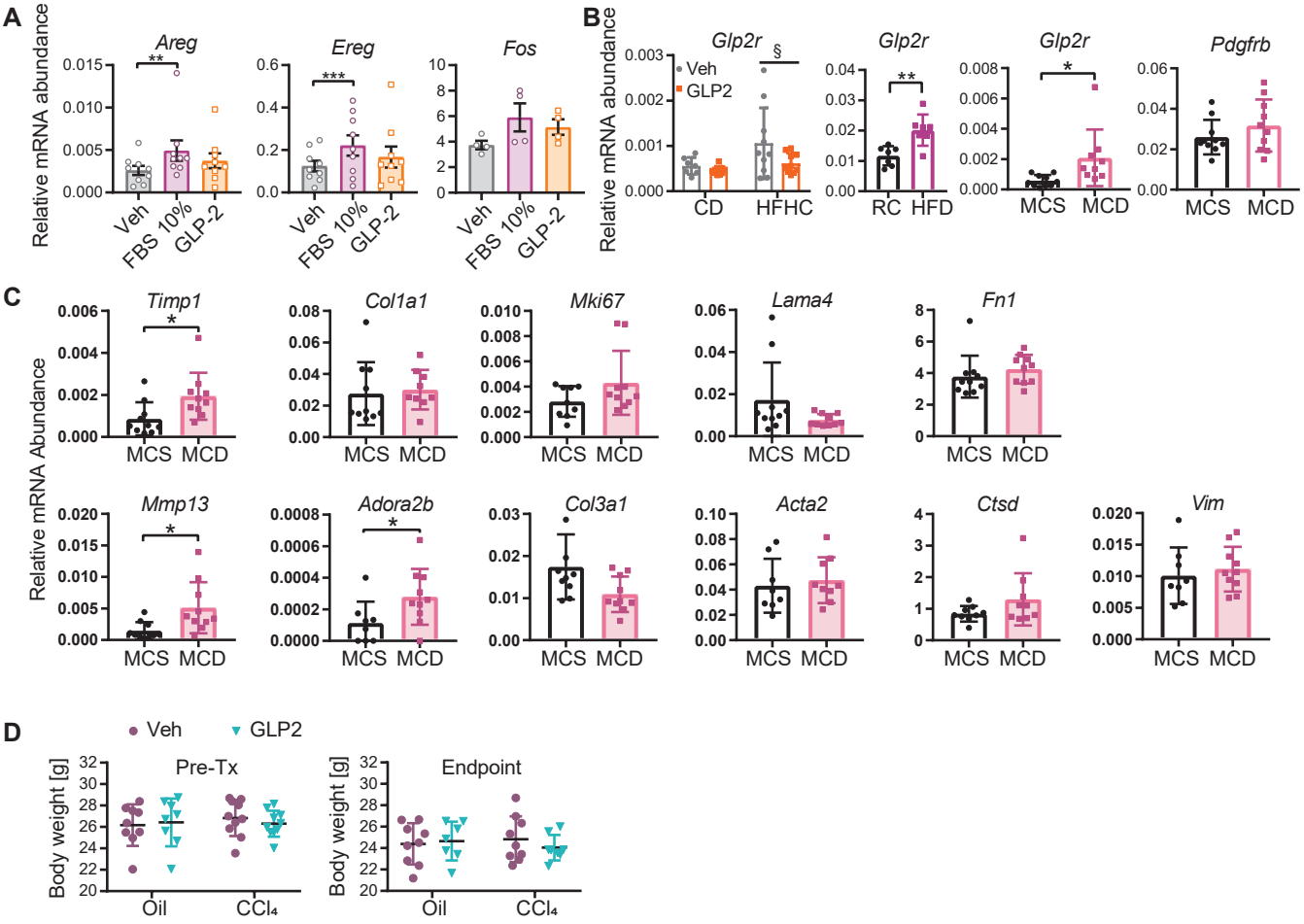
(A) Liver weight (left) normalized to tibia length (right). (B) Body weight. (C) Fasting plasma DPP-4 activity. (D) Fasting plasma active GLP-1 levels. (E) Fasting plasma triglyceride (TG) levels. (F) Plasma TG levels during an oral lipid tolerance test (LTT) and area under curve (AUC) data. (G) Plasma active GLP-1 levels at time 0, and 10min of the LTT in F. Data in (A-G) are from 8-12 week old *Glp2r*$^{+/+}$ and *Glp2r*$^{-/-}$ mice maintained on a RC diet (n=8/group) that were fasted for 5-6 hours prior to analyses. (H) Hepatic mRNA abundance, relative to *Ppia*, of the bile acid sensing receptors *Fxr* and *Tgr5* in *Glp2r*$^{+/+}$ and *Glp2r*$^{-/-}$ mice fed either CD or HFHC diet (n=9-10/group) as described in Figure 3A. Data are presented as the means ± SD. *p<0.05, ****p<0.0001 using two-way ANOVA with Tukey correction for multiple comparisons (panels A,H) or Student's t-test (panels B-G).



Supplemental Figure S3

Supplemental Figure S3 Fractionation of hepatocytes and non-parenchymal cells (NPC) from whole liver.

(A) mRNA expression of cell type-specific markers in whole liver (Liv, n=6), hepatocyte (Hep, n=10), and non-hepatocyte (NPC, n=4) fractions from male wild-type C57BL/6 mice 8-14 weeks old (n=3 for all panels). (B) Representative scatter plots of NPC fractions from 3 wild-type C57BL/6 male mice, FACS sorted by surface markers enriched for specific subpopulations. Top: $CD31^+$ (endothelial cells); KC $CD45^+$ (hematopoietic lineage cells with autofluorescence in the FITC range, corresponding to Kupffer cells (KC)); CD 45^+ (all other hematopoietic lineage cells); -/- ($CD31^-/CD45^-$ cells). Bottom: $CD31^-/CD45^-$ were further sorted to $CD26^+$ and $CD26^-$ fractions. CD26, also known as dipeptidyl peptidase-4 (DPP4), is expressed in most NPC and absent in HSCs. (C) mRNA abundance of cell-type-specific markers (top panels) and *Glp2r* (bottom panel) in Liv, whole liver; Hep, hepatocytes; Hep $CD26^+$, hepatocytes further purified by sorting to include $CD26^+$ cells; Kupffer, $CD45^+$ gated Kupffer cells from 3 wild-type C57BL/6 male mice. CD31-/CD45-/CD26- fraction corresponding to hepatic stellate cells (HSC) yielded too few cells for mRNA analysis and is not represented. *Cd11b*, hematopoietic marker enriched in both $CD45^+$ and Kupffer gates; *Spic*, Kupffer cell marker; *Cd31*,endothelial marker; *Dpp4*, enriched in hepatocytes and endothelial cells.



Supplemental Figure S4

(A) mRNA abundance, relative to *Ppia*, of immediate early genes (IEGs) used as markers of GLP-2 action in isolated hepatic stellate cells (HSC, isolated as described in Figures 5C and S3B). As described in Figure 5H, HSC were purified from 18 C57BL/6 WT mice 4-6 months old. Cells, combined from 1-4 mice per data point were incubated for 20min with either 10% fetal bovine serum (FBS), GLP-2 (50nM), or saline (Veh). Data are presented as the means ± SEM. *p<0.05, **p<0.01 using ratio-paired t-test. (B) Glp2r mRNA expression, relative to Ppia, in liver from mice exposed to various diets. From left to right: seventeen weeks of CD or HFHC diet as per Figure 1A (n=9-10/group), 12 weeks of regular chow (RC) or HFD (n=7/group), Glp2r and Pdgfrb (stellate cell marker) expression after 2 weeks of methionine choline sufficient (MCS) or deficient (MCD) diet, bottom panels, (n=9-10/group). Data are presented as the means ± SD. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using two-way ANOVA with Tukey correction for multiple comparisons or Student's t-test where appropriate.(C) mRNA abundance, normalized to *Ppia*, of HSC activation markers in whole liver. Wild-type mice (n=10/diet) were fed a methionine-choline deficient (MCD) or sufficient (MCS) diet for 2 weeks. Data are presented as the means ± SD. *p<0.05 by Student's t-test. (D) Body weights before (Pre-Tx) and at termination (Endpoint) in vehicle (Veh) or GLP-2 treated wild-type mice that received CCl_4 or control (Oil) treatment (n=8-11/group), as described in Figure 7A.

Supplemental Materials and methods

Reagent or resource	Source	Identifier
Chemicals, peptides and recombinant proteins		
Hepatocyte Wash medium	ThermoFisher	17704024
Liver Perfusion Medium	ThermoFisher	17701038
Liver Digest Medium	ThermoFisher	17703034
DNase I grade II	Sigma-Aldrich	10104159001
Pronase	Sigma-Aldrich	P5147
Collagenase D	Sigma-Aldrich (Roche)	11088866001
Tissue-Tek O.C.T compound	Sakura	4583
Mineral oil	Sigma-Aldrich	M1180
Olive oil	Sigma-Aldrich	O1514
Carbon tetrachloride	Toronto research chemicals	C176905
Nycodenz AG	Alere technologies	1002424
7-AAD	BD Pharmingen	559925
DMEM 4.5g/L glucose	Wisent Bioproducts	319-005-CL
Fetal Bovine Serum	Wisenet Bioproducts	080-150
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A7030
Charcoal Dextran	Sigma-Aldrich	C6241
PE anti-mouse CD26 (DPP-4) Ab	Biolegend	137804
APC anti-mouse CD45.2 Ab	Biolegend	109814
FITC anti-mouse CD31 Ab	Biolegend	102406
Teduglutide	Shire Pharmaceutical inc.	7001450

Critical commercial assays		
Mouse Proinflammatory Panel assay kit	Mesoscale	MSD V-PLEX K15048G
Active GLP-1 (ver. 2) kit	Mesoscale	K150JWC-2
Triglyceride assay kit (for plasma TG relating to Figure S2F)	Roche Diagnostics	11877771 216
Triglyceride calibrator (for plasma TG relating to Figure S2F)	Wako	464-01601
H-Gly-Pro-AMC HBr (for DPP-4 activity assay)	Bachem	I-1225
AMC (for DPP-4 activity assay)	Bachem	Q-1025
Protein assay kit II	Biorad	5000002
Triglyceride determination kit (For liver TG content)	**COBAS**	**7193130**
Triglyceride calibrator (For liver TG content)	COBAS	10759350
Cholesterol E kit (For liver cholesterol content)	Wako Diagnostics	999-02601
Experimental models: organisms, strains		
C57BL/6J WT mice	Jackson Laboratories	000664
***Glp2r* Null**	In-house colony at the Toronto Centre for Phenogenomics	Lee, SJ Endocrinology 2012 PMID 2225342

Diets

Regular Chow diet (RC)	Harlan Teklad (Envigo)	2018
10% fat control diet	Research Diets	D09100304
45% high fat diet	Research Diets	D12451i
60% high fat diet	Research Diets	D12492
HFHC diet	Research Diets	D09100301
Methionine-Choline deficient diet (MCD)	Cedarlane	A02082002Bi
Methionine-Choline sufficient diet (MCS)	Cedarlane	A02082003Bi

Software and algorithms

GraphPad Prism, versions 7, 8.3	GraphPad Prism software	https://www.graphpad.com; RRID:SCR_002798
ImageJ	NIH	1.52A
OlyVIA 2.9	OLYMPUS (Build 13735)	www.olympus-sis.com
Aperio ImageScope v12.3.2.8013	Leica Biosystems	https://aperio-imagescope.software.informer.com/12.3/

Others

Human Liver total RNA	Life Tech	AM7960
Human Liver total RNA	Agillient	540017
Human Liver RNA	Clontech	636531
Oligonucleotides		

acetyl-Coenzyme A dehydrogenase, medium chain. (*Acadm*)	Thermo Scientific	Mm00431611_m1
acyl-CoA synthetase long-chain family member 1 (*Acsl1*)	Thermo Scientific	Mm00484217_m1
actin, beta (*Actb*)	Thermo Scientific	Mm00607939_s1
adenosine A2b receptor (*Adora2b*)	Thermo Scientific	Mm00839292_m1
apolipoprotien A5 (*Apoa5*)	Thermo Scientific	Mm00475480_m1
apolipoprotein B (*ApoB*)	Thermo Scientific	Mm01545159_m1
apolipoprotien C3 (*Apoc3*)	Thermo Scientific	Mm00445670_m1
apolipoprotein E (*Apoe*)	Thermo Scientific	Mm00437573_m1
amphiregulin (*Areg*)	Thermo Scientific	Mm00437583_m1
bone morphogenetic protein 4 (*Bmp4*)	Thermo Scientific	Mm00432087_m1
c1q and tumor necrosis factor related protein 1 (*C1qtnf1*)	Thermo Scientific	Mm00480204_m1
chemokine (C-C motif) ligand 2 (*Ccl2*)	Thermo Scientific	Mm00441242_m1
CD14 antigen (Cd14)	Thermo Scientific	Mm00438094_g1
platelet/endothelial cell adhesion molecule 1 (*Pecam1*) (CD31)	Thermo Scientific	Mm01242584_m1
CD36 antigen (*Cd36*)	Thermo Scientific	Mm00432403_m1
CD68 antigen (*Cd68*)	Thermo Scientific	Mm00839636_g1
collagen, type I, alpha 1(*Col1a1*)	Thermo Scientific	Mm00801666_g1
collagen, type III, alpha 1(*Col3a1*)	Thermo Scientific	Mm01254476_m1
collagen, type VIII, alpha	Thermo Scientific	Mm01344184_m1

1(*Col8a1*)		
carnitine palmitoyltransferase 1a, liver (*Cpt1a*)	Thermo Scientific	Mm01231183_m1
carnitine palmitoyltransferase 2 (*Cpt2*)	Thermo Scientific	Mm00487205_m1
C-reactive protein, pentraxin-related (*Crp*)	Thermo Scientific	Mm00432680_g1
cathepsin D (*Ctsd*)	Thermo Scientific	Mm00515586_m1
chemokine (C-X-C motif) ligand 1 (*Cxcl1*)	Thermo Scientific	Mm00433859_m1
chemokine (C-X-C motif) ligand 12 (*Cxcl12*)	Thermo Scientific	Mm00445553_m1
chemokine (C-X-C motif) receptor 2 (*Cxcr2*) (IL8Rb)	Thermo Scientific	Mm00438258_m1
cytochrome P450, family 7, subfamily a, polypeptide 1 (*Cyp7a1*)	Thermo Scientific	Mm00484152_m1
diacylglycerol O-acyltransferase 1 (*Dgat1*)	Thermo Scientific	Mm00515643_m1
dipeptidyl peptidase4 (*Dpp4*)	Thermo Scientific	Mm00494538_m1
early growth response 1 (*Egr1*)	Thermo Scientific	Mm00656724_m1
elastase, neutrophil expressed (*Elane*)	Thermo Scientific	Mm00469310_m1
epiregulin (*Ereg*)	Thermo Scientific	Mm00514794_m1
fatty acid synthase (*Fasn*)	Thermo Scientific	Mm00662319_m1
fibronectin 1 (*Fn1*)	Thermo Scientific	Mm01256744_m1
FBJ osteosarcoma oncogene (Fos)	Thermo Scientific	Mm00487425_m1
nuclear receptor subfamily 1, group H, member 4 (BAR) (FXR)	Thermo Scientific	Mm01240553_m1

(*Nr1h4*)		
glucagon-like peptide 2 receptor (*Glp2r*) Mm01329475_m1	Thermo Scientific	Mm01329475_m1
glycerol-3-phosphate acyltransferase, mitochondrial. (*Gpam*)	Thermo Scientific	Mm00833328_m1
glutathione S-transferase, mu 3 (*Gstm3*)	Thermo Scientific	Mm00833923_m1
hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit (*Hadhb*)	Thermo Scientific	Mm00523880_m1
3-hydroxy-3-methylglutaryl-Coenzyme A reductase (*Hmgcr*)	Thermo Scientific	Mm01282499_m1
interferon gamma (*Ifng*)	Thermo Scientific	Mm01168134_m1
interleukin 10 (*Il10*)	Thermo Scientific	Mm99999062_m1
interleukin 18 (*Il18*)	Thermo Scientific	Mm00434226_m1
interleukin 1 beta (*Il1b*)	Thermo Scientific	Mm01336189_m1
interleukin 23, alpha subunit p19 (*Il23a*)	Thermo Scientific	Mm01160011_g1
interleukin 6 (*Il6*)	Thermo Scientific	Mm00446190_m1
interleukin 6 receptor, alpha (*Il6ra*)	Thermo Scientific	Mm00439653_m1
laminin, alpha 4 (*Lama4*)	Thermo Scientific	Mm01193660_m1
lecithin cholesterol acyltransferase (*Lcat*)	Thermo Scientific	Mm01247340_m1
low density lipoprotein receptor	Thermo Scientific	Mm00440169_m1

(*Ldlr*)		
lipase, hepatic (*Lipc*)	Thermo Scientific	Mm00433975_m1
lysyl oxidase (*Lox*)	Thermo Scientific	Mm00495386_m1
lipoprotein lipase (*Lpl*)	Thermo Scientific	Mm00434770_m1
macrophage receptor with collagenous structure (*Marco*)	Thermo Scientific	Mm00440265_m1
antigen identified by monoclonal antibody Ki 67 (*Mki67*)	Thermo Scientific	Mm01278617_m1
matrix metallopeptidase 13 (*MmP13*)	Thermo Scientific	Mm00439491_m1
MX dynamin-like GTPase 1 (*Mx1*)	Thermo Scientific	Mm00487796_m1
nuclear receptor subfamily 4, group A, member 1 (*Nr4a1*)	Thermo Scientific	Mm01300401_m1
2'-5' oligoadenylate synthetase 2 (Oas2)	Thermo Scientific	Mm00460961_m1
platelet derived growth factor receptor, beta polypeptide (*Pdgfrb*)	Thermo Scientific	Mm00435546_m1
pleckstrin homology-like domain, family A, member 1 (*Phlda1*)	Thermo Scientific	Mm00456345_g1
eukaryotic translation initiation factor 2-alpha kinase 2 PKR (*Eif2ak2*)	Thermo Scientific	Mm01235643_m1
patatin-like phospholipase domain containing 3 (*Pnpla3*)	Thermo Scientific	Mm00504420_m1
peroxisome proliferator activator receptor gamma (*Pparg*)	Thermo Scientific	Mm01184322_m1
Cyclophilin (*Ppia*)	Thermo Scientific	Mm02342430_g1
protein tyrosine phosphatase,	Thermo Scientific	Mm01293577_m1

receptor type, C (*Ptprc*) (CD45)		
RAS, dexamethasone-induced 1 (*Rasd1*)	Thermo Scientific	Mm00842185_g1
stearoyl-Coenzyme A desaturase 1 (*Scd1*)	Thermo Scientific	Mm00772290_m1
superoxide dismutase 2, mitochondrial (*Sod2*)	Thermo Scientific	Mm00449726_m1
Spi-C transcription factor (*Spic*)	Thermo Scientific	Mm00488428_m1
sterol regulatory element binding transcription factor 1 (*Srebp1c, Srebf1*)	Thermo Scientific	Mm00550338_m1
G protein-coupled bile acid receptor 1 (*Tgr5*) (GPBAR1)*	Thermo Scientific	Mm04212121_s1
tissue inhibitor of metalloproteinase 1 (*Timp1*)	Thermo Scientific	Mm00441818_m1
toll-like receptor4 (*Tlr4*)	Thermo Scientific	Mm00445273_m1
toll-like receptor8 (*Tlr8*)	Thermo Scientific	Mm04209873_m1
Tumor necrosis factor alpha (*Tnfa*)	Thermo Scientific	Mm00443258_m1
ubiquitin specific peptidase 18 (*Usp18*)	Thermo Scientific	Mm01188805_m1
vimentin (*Vim*)	Thermo Scientific	Mm01333430_m1
very low density lipoprotein receptor (*Vldlr*)	Thermo Scientific	Mm00443298_m1
X-box binding protein 1 (*Xbp1*)	Thermo Scientific	Mm00457357_m1

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to the Lead Contact, Dr. Daniel J. Drucker (drucker@lunenfeld.ca).