

Blood glucose lowering nuclear receptor agonists only partially normalize hepatic gene expression in *db/db* mice

Michael Loffler, Martin Bilban, Mark Reimers, Werner Waldhäusl and Thomas M. Stulnig

Clinical Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University Vienna, Vienna, Austria (M.L., W.W., T.M.S.); CeMM - Center of Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria (M.L., W.W., T.M.S.); Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna and Ludwig Boltzmann Institute for clinical and experimental Oncology, Vienna, Austria (M.B.); Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, Maryland (M.R.)

Running Title

Blood Glucose Lowering Drugs and Hepatic Gene Expression

Corresponding author

Thomas M. Stulnig, Clinical Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria; e-mail : thomas.stulnig@meduniwien.ac.at

Number of

Text pages	23
Tables	2
Figures	6 + 3 Supplementary Figures
References	40

Number of words

Abstract	220
Introduction	333
Discussion	402

ABBREVIATIONS PPAR, peroxisome proliferator-activated receptor; QPCR, quantitative real-time reverse-transcriptase PCR; SREBP, sterol regulatory element-binding protein; UPR, unfolded protein response.

Section

Endocrine and Diabetes

ABSTRACT

Agonists of the nuclear receptors peroxisome proliferator-activated receptor (PPAR) γ , PPAR α , and liver X receptors (LXR) reduce blood glucose in type 2 diabetic patients and comparable mouse models. Since the capacity of these drugs to normalize hepatic gene expression is not known we compared groups of obese diabetic *db/db* mice treated with agonists for PPAR γ (rosiglitazone [Rosi]; 10 mg/kg/day), PPAR α (Wy 14643 [Wy]; 30 mg/kg/day), and LXR (T0901317 [T09]; 40 mg/kg/day) and from untreated non-diabetic littermates (*db/+*) by oligonucleotide microarrays and quantitative reverse transcriptase polymerase chain reaction. The 10-day treatment period of *db/db* mice with Rosi, Wy, and T09 altered expression of 300, 620, and 735 genes including agonist-specific target genes, respectively. However, from the 337 genes differentially regulated in untreated *db/+* vs *db/db* animals, only 34 (10%), 51 (15%) and 82 (24%) were regulated in the direction of the *db/+* group by Rosi, Wy and T09, respectively. Gene expression normalization by drug treatment involved glucose homeostasis, lipid homeostasis and local glucocorticoid activation. In addition, our data pointed to hitherto unknown interference of these nuclear receptors with growth hormone receptor gene expression and endoplasmic reticulum stress. However, many diabetes-associated gene alterations remained unaffected or were even aggravated by nuclear receptor agonist treatment. These results suggest that diabetes-induced gene expression is minimally reversed by potent blood glucose lowering nuclear receptor agonists.

INTRODUCTION

Modern anti-diabetic agents targeting nuclear receptors are designed to modulate blood glucose levels and gene expression on a molecular level. Transcriptional regulation by agonists of peroxisome proliferator-activated receptor (PPAR) γ , PPAR α and LXR comprise genes involved in gluconeogenesis, fatty acid metabolism and ketogenesis (Willson et al., 2000; Venkateswaran et al., 2000). PPAR γ is a critical transcription factor involved in energy balance and activated by well-established anti-diabetic drugs, the thiazolidinediones (Lehmann et al., 1995). Nuclear receptor agonist or fatty acid dependent activation of PPAR α promotes peroxisomal proliferation, hepatic fatty acid oxidation and the generation of ketone bodies thereby providing substrates for energy metabolism in peripheral tissues (Issemann and Green, 1990). LXR α , the predominant LXR parologue in liver (Repa et al., 2000) regulates intracellular cholesterol and bile acid metabolism as well as expression of sterol regulatory binding protein (SREBP)-1c, the major lipogenic transcription factor (Schultz et al., 2000; Repa et al., 2000). Activation of LXR is associated with down-regulation of key genes involved in hepatic gluconeogenesis (Stulnig et al., 2002a; Cao et al., 2003; Laffitte et al., 2003). Moreover, nuclear receptors can modulate each other's gene expression as shown for PPAR γ and LXR α (Ide et al., 2003; Seo et al., 2004) pointing to a close relation of their transcriptional regulations and metabolic function. Agonists of PPAR γ , PPAR α and LXR all decrease blood glucose concentrations in type 2 diabetes patients and/or comparable animal models (Lehmann et al., 1995; Guerre-Millo et al., 2000; Laffitte et al., 2003) by regulating gene expression. In order to address the question whether normalization of blood glucose levels by these nuclear receptor agonists is accompanied by normalized gene expression, we analyzed genome-wide hepatic gene expression profiles of diabetic *db/db* mice treated with nuclear receptor agonists and their untreated non-diabetic littermates and compared them with

JPET# 93831

those of untreated *db/db* mice. By providing a comprehensive overview of drug-induced changes in gene expression in obese diabetic mice our data revealed that reversal of diabetes-associated alterations in hepatic gene expression occurs only to a very limited extent.

MATERIAL AND METHODS

Animals. Male C57BL/KsJ-lepr^{db}/lepr^{db} diabetic (*db/db*) mice and their nondiabetic littermates (*db/+*) were purchased from Charles River Laboratories Inc. (Germany) at seven weeks of age and maintained under standard light (12h light/dark) and temperature conditions (23°C). During one week of acclimatization mice were provided with a low-fat standard rodent diet (<3% fat; N1324, Altromin, Germany) and water *ad libitum*.

Treatment. For the experiment, the low-fat standard diet was either mixed with vehicle alone (ethanol; untreated) or supplemented with 0.005% (w/w) PPAR γ agonist Rosiglitazone (Rosi; Avandia, Smithkline Beecham, PLC, Middlesex, U.K.; corresponding to approximately 10 mg/kg/day; Hori et al., 2002), 0.02% PPAR α agonist Wy-14.643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid; Wy; Sigma-Aldrich, St Louis, MO, U.S.A.; corresponding to approximately 30 mg/kg/day; Edvardsson et al., 1999) or 0.025% of the synthetic LXR agonist T0901317 (N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1(trifluoromethyl)-ethyl]phenyl]-benzenesulfonamide; T09; generously provided by Amgen Inc., formerly Tularik Inc.; corresponding to approximately 40 mg/kg/day; Stulnig et al. , 2002a) followed by extensive evaporation of ethanol. Four groups of *db/db* (untreated, Rosi, Wy, T09; n = 5) and one group of *db/+* mice (untreated; n = 8) were treated for 10 days.

Tissue and blood analyses. Mice were anesthetized with Isoflurane and sacrificed by neck dislocation after cardiac puncture. The liver was immediately cut into small homogenous regions, snap frozen in liquid nitrogen and kept at -80°C until isolation of total RNA. Blood samples were drawn from the tail vein before starting the experimental diets. EDTA plasma separated from cardiac blood was stored in aliquots at -20°C until further analyses. Blood glucose was measured by an automated analyzer (ALCYON 300i, Abbott Laboratories,

Illinois, U.S.A.) at the beginning and the end of the experiment. Plasma cholesterol and triglycerides were determined by the Alcyon 300i analyzer (Abbott Laboratories). Serum concentrations of non-esterified fatty acids were measured with the Wako FFA-kit (Wako chemicals, Richmond, VA, U.S.A.), insulin by ELISA (Linco Research, St. Charles, MO, U.S.A.). Liver triglycerides were determined following lipid extraction as described (Haemmerle, 2002) but by using a commercially available enzymatic reagent (Rolf Greiner Biochemica, Flacht, Germany). The study protocol was approved by the local ethics committee for animal experiments and the Guidelines for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes were followed.

RNA preparation. Total RNA was prepared by disrupting equivalent regions of approximately 50 mg of liver tissue from each animal per group in TRIzol reagent (Invitrogen) with a tissue homogenizer followed by RNA isolation according the manufacturer's instructions. Total RNA samples were checked for integrity by agarose gel electrophoresis and the 2100 bioanalyzer (Agilent, Palo Alto, CA). For microarray cDNA synthesis total RNA samples were repurified with RNeasy MinElute kit (Qiagen).

Microarray hybridization and data analysis. Ten µg of total RNA from each animal (n=3 per group) was transcribed into first strand cDNA by Superscript II (Invitrogen, Carlsbad, CA, U.S.A.) using T7-Oligo(dT) primers followed by second strand synthesis and repurification according to the manufacturer's protocols (all Affymetrix, Santa Clara, CA, U.S.A.). Following in vitro transcription, 15µg of labeled and fragmented cRNA from each individual sample were hybridized to U74Av2 GeneChips (12k) which were scanned using the GeneArray scanner and further analyzed with Microarray Suite version 5.0 software

according to the manufacturer's protocols (all Affymetrix, Santa Clara, CA, U.S.A.). Array quality criteria for all chips included control of expression report files for background, background noise, scale factor <2, internal control gene 3' to 5' ratio, and hybridization control ratios, respectively. Normalization was performed by global scaling to an average intensity of 100 arbitrary units. Gene abundances were estimated by robust multi-array analysis (Irizarry, 2003) using the probe-level modeling (affyPLM) package from Bioconductor (www.bioconductor.org). This algorithm provides for calculation of means and standard errors of logarithmically transformed estimates, reflecting the inconsistency among the different probes for the same gene. We used a strategy similar to analysis of variance and computed a consensus estimate for the variability among all groups as a basis for t-test statistics. The multiple comparisons problem was addressed by estimating the false discovery rate (FDR) in a simple manner as the ratio of the expected number of false positives (resulting from permutation analyses with samples being randomly assigned to different groups) at a given *p*-value threshold to the number of positives actually found (Irizarry, 2003; Storey and Tibshirani, 2003). Using this statistical approach comparisons of gene expression with absolute fold changes of at least 1.5-fold (increase or decrease) were selected at *p*<0.01 as a default and *p*<0.001 where indicated. Abbreviation, annotation and analyses of genes meeting the selection criteria were done by combining available information from Affymetrix, Applied Biosystems (PANTHER), Jax, Genelynx, Kegg, Ensembl, Swiss-Prot and PubMed in a Filemaker Pro database.

Hierarchical clustering analysis. Genes altered by treatment with all nuclear receptor agonists by at least 1.5-fold (in either direction) and at *p* < 0.01 between mean gene expression and untreated *db/db* animals were selected for a cluster analysis. The genes were clustered using a distance measure defined as 1 minus the correlation between gene pair

measures, and using complete-linkage hierarchical clustering. The results are shown in Supplementary Figure 2.

Quantitative Real-Time Polymerase Chain Reaction. One µg of total RNA from each animal (n=5 per group) was treated with DNase I and reverse transcribed into cDNA by Superscript II using random hexamer priming (all Invitrogen, Carlsbad, CA, U.S.A.). Quantitative real-time polymerase chain reaction (QPCR) was performed for all samples per group using gene-specific FAM-TAMRA-labeled commercial Assays-on-Demand (Assay ID: Mm00839363_m1, G6pc; Mm00484574_m1, G6pt1; Mm00440636_m1, Pck1; Mm00662319_m1, Fasn; Mm00772290_m1, Scd1; Mm00833328_m1, Gpam; Mm00483985_m1, Crat; Mm00451571_m1, Slc25a20; Mm00443579_m1, Acox1; Mm00470091_s1, Ehhadh; Mm00478137_m1, Pex11a; Mm00550050_m1, Hmgcs2; Mm00476182_m1, Hsd11b1; Mm00439093_m1, Ghr; Mm00439561_m1, Igf1; Applied Biosystems, Foster City, CA, U.S.A.) or self-designed primer/probe combinations (SREBP-1c; ref. Stulnig et al., 2002b) normalized to 18S VIC-TAMRA labeled endogenous control (Applied Biosystems). Expression of specific mRNAs was quantitated in duplicates with a tolerated variance of ≤10% in an ABI PRISM 7000 Cycler (Applied Biosystems).

Statistics and calculations. Data are given in means ± S.E.M. unless indicated otherwise. Study groups were compared to untreated *db/db* mice by univariate ANOVA using Dunnett's t-test for post-hoc analysis except for evaluation of gene expression profiles (see above). Data from quantitative PCR and microarrays as well as other data exhibiting inequality of variances between groups according to Levene's test were log-transformed before ANOVA. The effect of diabetes was evaluated by comparing *db/+* to *db/db* mice resulting in its reciprocal (diabetes^{-1}) in order to facilitate direct comparisons with the normalizing effect of the compounds.

RESULTS

Metabolic data. This study was performed to elucidate to which extent blood glucose-lowering nuclear receptor agonists alter hepatic gene expression profiles of obese diabetic mice in convergence to non-diabetic animals. Gene expression profiles of *db/db* mice treated with agonists of PPAR γ (Rosi), PPAR α (Wy) and LXR (T09) and untreated *db/+* mice were compared to untreated diabetic mice. Treatment with each of the compounds resulted in reductions in blood glucose concentrations near to those of non-diabetic mice (Table 1). Plasma insulin concentrations were significantly increased only in the T09-treated group indicating that stimulation of insulin secretion as shown for cultured pancreatic islets and β -cells (Efanov, 2004) could also occur *in vivo*. Body weight remained constant during the treatment except a borderline ($p = 0.088$) increase in the Rosi group. The Wy and particularly T09-treated animals showed significant hepatomegaly with increased liver to body weight ratio by 1.8 and 2.5-fold, respectively, due to the known development of hepatic steatosis by T09 as revealed by highly elevated hepatic triglyceride contents in T09-treated *db/db* mice (Table 1).

Gene expression profiling. Gene expression profiles were evaluated using 12k oligonucleotide microarrays for 3 individual mice of each group and robust multiarray analysis. A p -value of < 0.01 was predefined together with a fold change of $\geq 1.5 / \leq -1.5$ -fold as selection criteria for profile comparisons including detection of functional groups. 337 genes were altered in untreated diabetic vs. non-diabetic mice (Table 2). Drug treatment of *db/db* mice elicited significant changes in the expression of a comparable number of genes, namely 300, 620, and 735 genes for Rosi, Wy, and T09, respectively. Estimated false discovery rates (FDR) were between 6-11% for $p < 0.01$ but about 80% of genes were altered at a $p < 0.001$ with FDR of 1-3%, respectively, indicating excellent confidence of data. Moreover, standardized logarithmic expression estimates from microarrays highly correlated

with those from quantitative PCR (adjusted $r^2 = 0.854$, $p < 0.0005$) indicating valid relative quantitation by microarray hybridization (data not shown).

We used the reciprocal form of the diabetes effect (diabetes^{-1}), i.e. untreated non-diabetic vs. diabetic mice, in order to facilitate direct comparison with treatment effects. Treatment-induced normalization of gene expression was defined as genes that were significantly changed by a compound in the same direction as diabetes^{-1} , i.e. in convergence to $db/+$ animals, without testing whether the gene actually reached the level of non-diabetic mice. From the genes (microarray probe sets) altered in db/db vs $db/+$ mice, only 34 (10% of all genes altered in diabetes^{-1}), 51 (15%) and 82 (24%) were normalized by Rosi, Wy and T09, respectively (Fig. 1A). In total, only a set of 19 genes (6%) were normalized by all blood glucose lowering drugs (listed in Supplementary Figure 1A) indicating that these genes could be particularly important in mediating the glucose lowering effects. These genes included some with clear implication in glucose metabolism and diabetes such as those involved in gluconeogenesis (e.g., glucose-6-phosphatase, fructose-1,6-bisphosphatase) and the glucocorticoid-activating enzyme 11 β -hydroxysteroid dehydrogenase type 1, whereas the functional implication of others for glucose lowering have to be assessed in detail. However, 56 (17%), 85 (25%) and 59 (18%) genes altered in db/db vs $db/+$ mice were regulated by Rosi, Wy and T09, respectively, in the direction opposite to diabetes^{-1} (Fig. 1B). The 30 genes (probe sets) regulated by all compounds in the direction opposite to diabetes^{-1} (listed in Supplementary Figure 1B) thus possibly giving insight into augmented adaptive processes, e.g., comprised genes involved in mitochondrial and peroxisomal β -oxidation (Acaa1, Dci, Ehhadh), including the important peroxisomal biogenesis factor (Pex11a) as discussed in detail below. Treatment with nuclear receptor agonists regulated many genes not altered by diabetes^{-1} itself and resulted in considerably overlaps in gene expression profiles in diabetic mice (Fig. 1C) similar to that shown for PPAR α , LXR, and retinoid X receptor in non-diabetic mice (Anderson et al., 2004). The overlap of PPAR γ agonist treatment with other

compounds is noteworthy, since PPAR γ is only weakly expressed in liver and thus direct crosstalk between nuclear receptors cannot be accounted for. Supplementary Figure 2 provides a tree obtained by hierarchical clustering of genes altered in parallel by all nuclear receptors agonists to highlight the correlation of their regulation. A complete list of all genes altered in *db/db* vs *db/+* mice (shown as diabetes⁻¹) or by treatment with any compound is given in Supplementary Figure 3.

Gluconeogenesis. Hepatic gluconeogenesis and glucose output is significantly enhanced in type 2 diabetes patients and comparable mouse models and contributes to high blood glucose levels. Treatment with each of the blood glucose lowering nuclear receptor agonists resulted in normalization of gluconeogenetic key enzyme gene expression as shown for phosphoenol pyruvate carboxykinase 1 (PEPCK; gene Pck1; 1.2 fold; $p < 0.05$), fructose-1,6-bisphosphatase (Fbp1) and the catalytic and transport units of glucose-6-phosphatase (G-6-Pase; G6pc and G6pt1) (Fig. 2). Interestingly, the PPAR α agonist treatment led to an increased expression of fructose-2,6-bisphosphatase (Fbp2) which could imply an additional regulation of Fbp1 through competitive inhibition by fructose-2,6-bisphosphate. Expression of aldolase B (Aldo2) that catalyzes cleavage of fructose-1,6-bisphosphate and functions as a feed forward activator of pyruvate kinase was normalized by both Wy (-1.8 fold; $p < 0.002$) and Rosi (-1.4 fold; $p = 0.042$). Glucokinase (Gck) whose expression was increased by 1.4 fold in diabetes⁻¹, was normalized in the Rosi group (1.4 fold; $p = 0.003$) and T09 group (1.8 fold; $p < 0.001$). Additional transfer of glucose-6-phosphate through the pentose phosphate shunt in the LXR-treated group is indicated by elevated expression of Gck, X-linked glucose-6-phosphate dehydrogenase (G6pdx), glucose-6-phosphate dehydrogenase 2 (G6pd2), ribose 5-phosphate isomerase A (Ripa) and transketolase (Tkt; Supplementary Figure 3). In general these data indicate that treatment of *db/db* mice with different blood glucose lowering nuclear receptor agonist resulted in normalized expression of key genes involved in glucose homeostasis even though some genes were regulated in an agonist-specific manner.

Lipogenesis. Hepatic lipogenesis and fatty acid desaturation has been implicated in various pathological conditions including obesity and diabetes (Ntambi et al., 2002). Hepatic gene expression of lipogenic enzymes, e.g., stearoyl-Coenzyme A desaturase 1 (Scd1), was generally increased in *db/db* vs *db/+* animals (Fig. 3A). Treatment of *db/db* mice with any blood glucose lowering compound led to a further strong increase of Scd1 gene expression (Fig. 3A). Scd1 expression is regulated by sterol regulatory element-binding protein-(SREBP)-1c-dependent and independent mechanisms (Miyazaki et al., 2004). Since induction of Scd1 by drugs correlated with elevated SREBP-1c expression levels only in the T09 group (Fig. 3A), Scd1 upregulation by PPAR agonists appears to occur predominantly by SREPB-1c-independent mechanisms. Parallel to Scd1 expression, fatty acid synthase (Fasn) and glycerol-3-phosphate acyltransferase (Gpam) was strongly induced by treatment with any agonist whereas expression of hepatic lipase was decreased (Fig. 3A). Thereby, the nuclear receptor agonists aggravated diabetes-associated alterations in lipogenesis in parallel with their glucose lowering effect (Way et al., 2001). Such regulation opposite to diabetes⁻¹ could on the one hand indicate enhancement of adaptive processes that were already induced by diabetes itself but also worsening of detrimental diabetes-associated alterations. Recent data suggest that upregulation of lipogenesis provides an effective means to inhibit diabetes development by shifting the lipogenic burden from adipose tissue to the liver (Nadler and Attie, 2001). Altogether alterations in hepatic lipid homeostasis provoked hepatic triglyceride accumulation particularly in the T09-treated and – to a lesser extent – in Rosi-treated animals. Moreover, the LXR agonist increased blood plasma triglyceride concentrations, whereas Rosi and Wy lowered plasma triglyceride concentrations (Table 1; ref. Chisholm et al., 2003; Oakes et al., 1994). In contrast to lipogenic enzymes, genes involved in cholesterolgenesis such as 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (Hmgr) and synthase 1 (Hmgcs1), isopentenyl-diphosphate delta isomerase (Idi1), farnesyl diphosphate farnesyl transferase 1 (Fdft1) and NAD(P)-dependent steroid dehydrogenase-like (Nsdlh) were not

altered by diabetes and were differently changed by nuclear receptor agonists (Supplementary Figure 3. and data not shown).

Mitochondrial and peroxisomal β -oxidation. β -oxidation of free fatty acids provides energy and substrates for ketogenesis. Expression of genes implicated in mitochondrial fatty acid import including the carnitine acyltransferase Crat and the translocase Slc25a20 (Ramsay et al., 2001; Sekoguchi et al., 2003). Expression of both genes was significantly increased in diabetic vs. non-diabetic mice and further elevated by treatment with blood glucose-lowering nuclear receptor agonists indicating augmented mitochondrial fatty acid import (Fig. 3B). Expression of the long chain-specific carnitine acyltransferases (Cpt1a, Cpt2) was increased by diabetes and further elevated in response to Wy treatment. Expression of enzymes involved in mitochondrial fatty acyl oxidation such as dodecenoyl-Coenzyme A delta isomerase (Dci) and t acetyl-CoA dehydrogenases (Acads, Acadm, Acadl) was somewhat increased in diabetic animals and further elevated by nuclear receptor agonists (Fig. 3C and Supplementary Figure 3). Peroxisomes oxidize fatty acids by different pathways with preference for long chain and very long chain fatty acids (Van Veldhoven and Mannaerts, 1999). Expression of enzymes of the classical pathway, namely acyl-Coenzyme A oxidase 1 (Acox1), enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A dehydrogenase (Ehhadh) and acetyl-Coenzyme A acyltransferase 1 (Acaa1) was more pronounced in *db/db* vs. *db/+* animals (Lan et al., 2003) and further increased by nuclear receptor agonist treatment, particularly Wy (Fig. 3C) in parallel with peroxisomal biogenesis factor 11a (Pex11a; Fig. 3C). Notably, hepatic triglyceride accumulation particularly in T09-treated animals revealed that increased β -oxidation did not sufficiently counteract elevated lipogenesis to prevent hepatic steatosis. Increased ketogenesis in diabetes was indicated by elevated expression of key genes 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgcs2) and 3-hydroxy-3-methylglutaryl-Coenzyme A lyase (Hmgcl; Fig. 3D). Particularly PPAR agonists further increased Hmgcl and Wy also increased Hmgcs2 expression suggesting increased

ketogenesis. In conclusion, treatment with different blood glucose-lowering nuclear receptor agonists resulted in increased expression of most genes contributing to mitochondrial and peroxisomal fatty acid oxidation and ketogenesis thereby aggravating the diabetes-associated shift to fatty acid metabolism.

Glucocorticoid activation. Type 2 diabetes has been shown to be associated with alterations in local activation of inactive glucocorticoid precursors (cortisone, 11-dehydro corticosterone) to active glucocorticoids (corticosterone, cortisol) by 11 β -hydroxysteroid dehydrogenase type 1 (Hsd11b1). Hsd11b1 is particularly expressed in human and rodent liver and adipose tissue (Bujalska et al., 2002) and increased expression has been linked to development of diabetes and the metabolic syndrome (Masuzaki et al., 2001; Aoki et al., 2001). Treatment with agonists of the PPAR and LXR family has been shown recently to affect expression of Hsd11b1 in adipose tissue and liver (Stulnig et al., 2002b; Cao et al., 2003). Treatment of either nuclear receptor agonists diminished Hsd11b1 expression even below the level of non-diabetic mice (Fig. 4). Since oxo-reductase activity of Hsd11b1 depends on NADPH, mutations in hexose-6-phosphate dehydrogenase (H6pd) synergize with weak Hsd11b1 mutations in glucocorticoid activation (Draper et al., 2003). However, H6pd gene expression was not changed in this study (Fig. 4). Notably, Hsd11b1 was one of the only 19 transcripts that were normalized by all blood glucose lowering nuclear receptor agonists (Supplementary Fig. 1A). These data emphasize the importance of 11 β -hydroxysteroid dehydrogenase 1 for the development of insulin resistance and suggest the use of specific inhibitors of this enzyme in diabetes treatment.

Growth hormone signaling. Growth hormone (GH) antagonizes insulin signaling and elicits insulin resistance resulting in increased hepatic glucose production. In contrast to unchanged expression in diabetic vs. non-diabetic mice, treatment with blood nuclear receptor agonists downregulated growth hormone receptor gene (Ghr) expression (Fig. 5), whose abundance is related to GH tissue sensitivity (Dominici and Turyn, 2002; Iida et al., 2004), as

well as expression of insulin-like growth factor-1 (Igf-1), a prototype GH-responsive gene in the liver. These data indicate interference with GH action by nuclear receptor agonist treatment. Since inhibition of GH action improves insulin sensitivity (Yakar et al., 2004), this could be an additional mechanism how PPAR and LXR agonists ameliorate glucose homeostasis in diabetes.

Endoplasmic reticulum stress. Endoplasmic reticulum stress is a major contributor for the development of obesity and insulin resistance as shown very recently (Özcan et al., 2004). Obesity triggers an unfolded protein response (UPR) in liver that is controlled by X-box-binding protein-1 (XBP-1) (Yoshida et al., 2001). Notably, XBP-1^{+/−} mice are prone to insulin resistance due to impaired insulin signal transduction during endoplasmic reticulum stress (Özcan et al., 2004). Glucose-regulated/binding immunoglobulin protein Grp78 (Hspa5), an endoplasmic reticulum chaperone, and the protein kinase inhibitor p58^{ipk} (Dnajc3) are upregulated during UPR and the latter has been shown to be a XBP-1 target gene as is thioredoxin domain containing protein-7 (Txndc7) (Lee et al., 2003). XBP-1 expression was significantly lowered by all blood glucose-lowering nuclear receptor agonists (Fig. 6) in parallel with Grp78, Dnajc3 and Txndc7 pointing to reduced XBP-1 activity and amelioration of endoplasmic reticulum stress. Reduced expression of Grp78 in untreated *db/db* animals compared to lean mice could be secondary to the nearly twofold reduced expression of XBP-1 in untreated *db/db* vs. *db/+* mice. It is intriguing to speculate that the reduced expression of XBP-1 in *db/db* animals indicates a reduced capacity to deal with endoplasmic reticulum stress similar to that seen in XBP-1^{+/−} mice whereas further reduction of the expression of UPR genes by nuclear receptor agonist treatment of diabetic mice reflects a decline of endoplasmic reticulum stress. Thus, reduction of endoplasmic reticulum stress could be a novel mechanism how PPAR and LXR agonists lower blood glucose concentrations in diabetic mice prone to UPR by lowered XBP-1 expression.

DISCUSSION

Insulin-sensitizing compounds such as agonists for PPAR γ and PPAR α are widely used in clinical practice to ameliorate diabetes-induced alterations in glucose and lipid metabolism, respectively. In this study we show that although blood glucose lowering agonists for the nuclear receptors PPAR γ , PPAR α and LXR regulate expression of a large number of genes, they improve only some diabetes-associated alterations mainly by normalization of gluconeogenetic gene expression. A large number of diabetes-associated alterations in gene expression were not reversed towards levels found in non-diabetic mice but even deteriorated by drug treatment including genes implicated in lipogenesis, peroxisomal and mitochondrial function. Some of these regulations, e.g., the elevated expression of genes involved in lipogenesis, indicate that nuclear receptor agonists enhanced adaptive processes protecting obese mice from further metabolic derangements, e.g. by shifting the lipogenic burden to the liver. A gene expression study by itself cannot discriminate between causal and adaptive alterations. However, irrespective of whether diabetes-associated alterations by nuclear receptor agonist treatment were primarily of causal or adaptive nature, these changes indicate that a cure in a molecular sense, i.e., correction of causal diabetes-associated molecular alterations, has been achieved by the compounds only to a very limited extent. In addition, our study disclosed hints on possible novel modes of action how nuclear receptor agonists ameliorate blood glucose concentrations. Particularly interference with growth hormone signaling and reduction of endoplasmic reticulum stress warrant investigations in future focused studies. Moreover, the significantly increased insulin plasma concentration by T09 treatment revealed that LXR agonism or selective LXR modulation could provide a novel pharmacological approach to stimulate insulin secretion. However, issues of preventing hepatic steatosis by these compounds and possible β -cell lipotoxicity by increased SREBP-1c

JPET# 93831

expression (Efanov, 2004) have to be addressed first. Notably, the model for type 2 diabetes used here cannot discriminate between alterations induced by diabetes or obesity alone, respectively. Hence changes provoked by obesity but not by metabolic derangements cannot be overcome by the action of the compounds that did not induce weight loss. However, alterations induced by obesity and metabolic derangements are usually combined also in type 2 diabetes patients.

In conclusion, this study revealed that apart from pointing to novel modes of action, currently available blood glucose-lowering nuclear receptor agonists by far do not normalize diabetes-associated molecular alterations. Despite our advances during recent years, there is still a need for developing novel drugs for effective treatment of type 2 diabetes at the molecular level.

Acknowledgments. We thank Sylvia Molzer for technical assistance and Jelena Todoric for liver triglyceride determination.

REFERENCES

- Anderson SP, Dunn C, Laughter A, Yoon L, Swanson C, Stulnig TM, Steffensen KR, Chandraratna RAS, Gustafsson JA, Corton JC (2004) Overlapping transcriptional programs regulated by peroxisome proliferator-activated receptor alpha, retinoid X receptor and liver X receptor in the mouse liver. *Mol Pharm* **66**:1440-1452.
- Aoki K, Homma M, Hirano T, Oka K, Satoh S, Mukasa K, Ito S and Sekihara H (2001) mRNA and enzyme activity of hepatic 11beta-hydroxysteroid dehydrogenase type 1 are elevated in C57BL/KsJ-db/db mice. *Life Sci* **69**:2543-2549.
- Bujalska IJ, Walker EA, Tomlinson JW, Hewison M and Stewart PM (2002) 11Beta-hydroxysteroid dehydrogenase type 1 in differentiating omental human preadipocytes: from de-activation to generation of cortisol. *Endocr Res* **28**:449-461.
- Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, Zhang Y, Stayrook KR, Suen C, Otto KA, Miller AR, Dai J, Foxworthy P, Gao H, Ryan TP, Jiang XC, Burris TP, Eacho PI and Etgen GJ (2003) Antidiabetic action of a liver X receptor agonist mediated by inhibition of hepatic gluconeogenesis. *J Biol Chem* **278**:1131-1136.
- Chisholm JW, Hong J, Mills SA and Lawn RM (2003) The LXR ligand T0901317 induces severe lipogenesis in the db/db diabetic mouse. *J Lipid Res* **44**:2039-2048.
- Dominici FP and Turyn D (2002) Growth hormone-induced alterations in the insulin-signaling system. *Exp Biol Med (Maywood)* **227**:149-157.
- Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, Lavery GG, Bedendo O, Ray DW, Laing I, Malunowicz E, White PC, Hewison M, Mason PJ, Connell JM, Shackleton CH and Stewart PM (2003) Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet* **34**:434-439.
- Edvardsson U, Alexandersson M, Brockenhuis von Lowenhielm H, Nystrom AC, Ljung B, Nilsson F and Dahllof B (1999) A proteome analysis of livers from obese (ob/ob)

- mice treated with the peroxisome proliferator WY14,643. *Electrophoresis* **20**:935-942.
- Efanov AM, Sewing S, Bokvist K, Gromada J (2004) Liver X receptor activation stimulates insulin secretion via modulation of glucose and lipid metabolism in pancreatic beta-cells. *Diabetes* **53**:S75-78.
- Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulaire P, Derudas B, Herbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B (2000) Peroxisome proliferator activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* **275**:16638-16642.
- Haemmerle G, Zimmermann R, Strauss JG, Kratky D, Riederer M, Knipping G, Zechner R (2002) Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. *J Biol Chem* **277**:12946-12952.
- Hori H, Sasaoka T, Ishihara H, Wada T, Murakami S, Ishiki M and Kobayashi M (2002) Association of SH2-containing inositol phosphatase 2 with the insulin resistance of diabetic db/db mice. *Diabetes* **51**:2387-2394.
- Ide T, Shimano H, Yoshikawa T, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Yatoh S, Iizuka Y, Tomita S, Ohashi K, Takahashi A, Sone H, Gotoda T, Osuga J, Ishibashi S and Yamada N (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. II. LXRs suppress lipid degradation gene promoters through inhibition of PPAR signaling. *Mol Endocrinol* **17**:1255-1267.
- Iida K, Del Rincon JP, Kim DS, Itoh E, Nass R, Coschigano KT, Kopchick JJ and Thorner MO (2004) Tissue-specific regulation of growth hormone (GH) receptor and insulin-like growth factor-I gene expression in the pituitary and liver of GH-deficient (lit/lit) mice and transgenic mice that overexpress bovine GH (bGH) or a bGH antagonist. *Endocrinology* **145**:1564-1570.

- Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B and Speed TP (2003) Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* **31**:e15.
- Issemann I, Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**:645-650.
- Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, Castrillo A, Wilpitz DC, Mangelsdorf DJ, Collins JL, Saez E and Tontonoz P (2003) Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc Natl Acad Sci U S A* **100**:5419-5424.
- Lan H, Rabaglia ME, Stoehr JP, Nadler ST, Schueler KL, Zou F, Yandell BS and Attie AD (2003) Gene expression profiles of nondiabetic and diabetic obese mice suggest a role of hepatic lipogenic capacity in diabetes susceptibility. *Diabetes* **52**:688-700.
- Lee AH, Iwakoshi NN, Glimcher LH (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* **23**:7448-7459.
- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM and Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* **270**:12953-12956.
- Masuzaki H, Paterson J, Shinya H, Morton NM, Mullins JJ, Seckl JR and Flier JS (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science* **294**:2166-2170.
- Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ and Ntambi JM (2004) Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. *J Biol Chem* **279**:25164-25171.
- Nadler ST and Attie AD (2001) Please pass the chips: genomic insights into obesity and

- diabetes. *J Nutr* **131**:2078-2081.
- Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendzierski CM, Yandell BS, Song Y, Cohen P, Friedman JM and Attie AD (2002) Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A* **99**:11482-11486.
- Oakes ND, Kennedy CJ, Jenkins AB, Laybutt DR, Chisholm DJ and Kraegen EW (1994) A new antidiabetic agent, BRL 49653, reduces lipid availability and improves insulin action and glucoregulation in the rat. *Diabetes* **43**:1203-1210.
- Özcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**:457-461.
- Ramsay RR, Gandour RD and van der Leij FR (2001) Molecular enzymology of carnitine transfer and transport. *Biochim Biophys Acta* **1546**:21-43.
- Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL and Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRAalpha and LXRBeta. *Genes Dev* **14**:2819-2830.
- Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoelen M, Mangelsdorf DJ, Lustig KD and Shan B (2000) Role of LXR_s in control of lipogenesis. *Genes Dev* **14**:2831-2838.
- Sekoguchi E, Sato N, Yasui A, Fukada S, Nimura Y, Aburatani H, Ikeda K and Matsuura A (2003) A novel mitochondrial carnitine-acylcarnitine translocase induced by partial hepatectomy and fasting. *J Biol Chem* **278**:38796-38802.
- Seo JB, Moon HM, Kim WS, Lee YS, Jeong HW, Yoo EJ, Ham J, Kang H, Park MG, Steffensen KR, Stulnig TM, Gustafsson JA, Park SD and Kim JB (2004) Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor gamma expression. *Mol Cell Biol* **24**:3430-3444.

- Storey JD and Tibshirani R (2003) Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A* **100**:9440-9445.
- Stulnig TM, Steffensen KR, Gao H, Reimers M, Dahlman-Wright K, Schuster GU, Gustafsson JÅ (2002a) Novel roles of liver X receptors exposed by gene expression profiling in liver and adipose tissue. *Mol. Pharmacol.* **62**:1299-1305.
- Stulnig TM, Oppermann U, Steffensen KR, Schuster GU and Gustafsson JÅ (2002b) Liver X receptors downregulate 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. *Diabetes* **51**:2426-2433.
- Van Veldhoven PP and Mannaerts GP (1999) Role and organization of peroxisomal beta-oxidation. *Adv Exp Med Biol* **466**:261-272.
- Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA and Tontonoz P (2000) Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci U S A* **97**:12097-12102.
- Way JM, Harrington WW, Brown KK, Gottschalk WK, Sundseth SS, Mansfield TA, Ramachandran RK, Willson TM and Kliewer SA (2001) Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. *Endocrinology* **142**:1269-1277.
- Willson TM, Brown PJ, Sternbach DD and Henke BR (2000) The PPARs: from orphan receptors to drug discovery. *J Med Chem* **43**:527-550.
- Yakar S, Setser J, Zhao H, Stannard B, Haluzik M, Glatt V, Bouxsein ML, Kopchick JJ and LeRoith D (2004) Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1-deficient mice. *J Clin Invest* **113**:96-105.
- Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**:881-891.

JPET# 93831

FOOTNOTES

Title footnote: This work was supported by CeMM – Center of Molecular Medicine, a basic research institute within the companies of the Austrian Academy of Sciences (to T.M.S. and W.W.), and the Joseph Skoda Award of the Austrian Society for Internal Medicine (to T.M.S.).

Address reprint requests to Thomas M. Stulnig, Clinical Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria; e-mail : thomas.stulnig@meduniwien.ac.at

FIGURE LEGENDS

Fig. 1. Overlap of diabetes and drug effects. Out of the 337 genes (microarray probe sets) altered by diabetes figures in overlapping wheels of panels (A) and (B) indicate the number of genes that were also regulated by PPAR γ , PPAR α and LXR agonists, respectively, in direction to normalize (A) or deteriorate (B) diabetes-associated changes (see Supplementary Figure 1 and 2). Panel (C) gives total numbers of genes regulated by the compounds illustrating considerable overlap of drug effects. Numbers with connecting lines to the wheels indicate the total number of genes regulated by the respective nuclear receptor agonist. Note that only a minority of diabetes-associated gene regulations were normalized by the treatments, and that many genes not associated with diabetes-associated alterations were regulated by the compounds.

Fig. 2. Expression of genes involved in gluconeogenesis. Diabetic *db/db* mice were treated for 10 days with Rosi, Wy, or T09, respectively, or left untreated as were non-diabetic *db/+* mice. Gene expression was evaluated by QPCR (default; n=5) or by microarrays (indicated by black dot; n=3) and is given in percent of that in untreated *db/db* mice. G6pc, glucose-6-phosphatase, catalytic subunit; G6pt, glucose-6-phosphatase, transport subunit; PEPCK (Pck1), phosphoenolpyruvate carboxykinase 1, cytosolic; Fbp1, fructose bisphosphatase 1; Aldo2, aldolase 2, B isoform; ut, untreated. Significant differences compared to untreated *db/db* are indicated as follows: *, p < 0.05; †, p < 0.01; ‡, p < 0.001.

Fig. 3. Lipid homeostasis and ketogenesis. A) Expression of genes involved in lipogenesis. Gene expression from mice is given as detailed in legend to Fig. 2. Gene expression was evaluated by QPCR (default; n=5) or by microarrays (indicated by black dot; n=3) and is given in percent of that in untreated *db/db* mice. Fasn, fatty acid synthase; Scd1, stearoyl-

Coenzyme A desaturase 1; Gpam, glycerol-3-phosphate acyltransferase, mitochondrial; Dgat1, diacylglycerol O-acyltransferase 1; Lipc, hepatic lipase; Srebp-1c, sterol regulatory element binding factor 1; ut, untreated; geometric mean is given for columns exceeding scale of Y-axis. Significant differences compared to untreated *db/db* are indicated as follows: *, p < 0.05; †, p < 0.01; ‡, p < 0.001. **B)** Expression of genes involved in mitochondrial fatty acid import. Crat, carnitine acetyltransferase; Slc25a20, solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20; Cpt1a, carnitine palmitoyl- transferase 1, liver; Cpt2, carnitine palmitoyl- transferase 2. **C)** Expression of genes involved in mitochondrial and peroxisomal β -oxidation. Acox1, acyl-Coenzyme A oxidase 1, palmitoyl; Dci, dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenzyme A isomerase); Ehhadh, enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase; Acaa1, 3-ketoacyl-CoA thiolase B, acetyl-Coenzyme A acyltransferase 1; Pex11a, peroxisomal biogenesis factor 11a. **D)** Expression of genes involved in ketogenesis. Hmgcs2, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2; Hmgcl, 3-hydroxy-3-methylglutaryl-Coenzyme A lyase.

Fig. 4. Glucocorticoid metabolism. Gene expression from mice is given as detailed in legend to Fig. 2. Gene expression was evaluated by QPCR (default; n=5) or by microarrays (indicated by black dot; n=3) and is given in percent of that in untreated *db/db* mice. Hsd11b1, hydroxysteroid 11-beta dehydrogenase 1; H6pd, hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase); ut, untreated. Significant differences compared to untreated *db/db* are indicated as follows: ‡, p < 0.001.

Fig. 5. Growth hormone signaling. Gene expression from mice is given as detailed in legend to Fig. 2. Gene expression was evaluated by QPCR (default; n=5) or by microarrays (indicated by black dot; n=3) and is given in percent of that in untreated *db/db* mice. Ghr,

growth hormone receptor; Igf-1, insulin-like growth factor 1; ut, untreated. Significant differences compared to untreated *db/db* are indicated as follows: †, p < 0.01; ‡, p < 0.001.

Fig. 6. Expression of genes involved in endoplasmic reticulum stress. Gene expression from mice is given as detailed in legend to Fig. 2. Gene expression was evaluated by QPCR (default; n=5) or by microarrays (indicated by black dot; n=3) and is given in percent of that in untreated *db/db* mice. Xbp1, X-box binding protein 1; Grp78 (Hspa5), heat shock 70kD protein 5 (glucose-regulated protein); Dnajc3, DnaJ (Hsp40) homolog, subfamily C, member 3; Txndc7, thioredoxin domain containing protein-7; ut, untreated; *, p < 0.05; †, p < 0.01; ‡, p < 0.001.

Supplementary Figure 1. Genes regulated by all nuclear receptor agonists in the direction to normalize (A) or aggravate (B) diabetes-induced alterations. Gene Ontology (GO) identifiers were added as a functional annotation. Microarray probe sets representing identical genes show up repeatedly.

Supplementary Figure 2. Clustering of genes regulated by all nuclear receptor agonists. The profiles cluster according to the pattern across all conditions including alterations between untreated diabetic and non-diabetic animals. Genes downregulated by the compounds are represented in the upper part, upregulated genes in the lower part of the figure. Several different patterns of response are observed; for example the upmost group of the lower half corresponds to genes that are up-regulated in all conditions including the diabetes⁻¹ effect (i.e., *db/+* vs. *db/db* animals). On the other hand, the downmost genes of the upper half correspond to genes with increased expression in *db/+* vs. *db/db* animals but downregulated by all compounds.

Supplementary Figure 3. Hepatic gene expression profiles of drug-treated *db/db* and untreated *db/+* compared to untreated *db/db*. Diabetic *db/db* mice were treated for 10 days with Rosi (R), Wy (W), or T09 (T), respectively, or left untreated as were non-diabetic *db/+* mice. Probe sets numbered with affy ID# represent genes identified by genbank entry numbers (genbank ID#), gene symbols and names. The fold change difference to untreated *db/db* mice is given as a color code and in numbers. The mean estimate of expression in untreated *db/db* mice is related to the mean of all genes on each microarray that was set to 100.

TABLE 1. Animal characteristics and blood analyses

Parameter (units)	<i>db/db</i>				<i>db/+</i>
	untreated	Rosi	Wy	T09	untreated
Body weight (g)					
before treatment	42.7±1.8 ^a	42.6±1.6	42.0±1.7	42.5±1.3	27.3±1.4***
end of treatment	43.7±1.7	46.7±1.9	42.3±2.7	43.4±2.4	27.9±1.4***
Liver weight (g)	2.30±0.30	2.68±0.35	3.83±0.36***	5.67±0.29***	1.43±0.13***
Liver/body weight (%)	5.2±0.5	5.7±0.6	9.1±0.9***	13.1±0.2***	5.1±0.3
Blood glucose ^b (mmol/l)					
before treatment	23.4±3.2	19.5±5.1	24.5±3.7	25.0±4.8	8.1±0.9***
end of treatment	28.7±2.0	10.4±1.7***	14.7±4.5***	11.9±4.0***	7.9±0.7***
Insulin ^c (nmol/l)	0.52±0.26	0.53±0.26	0.41±0.22	1.75±0.58***	0.37±0.19
Triglycerides ^c (mmol/l)	2.3±0.5	0.7±0.3***	1.1±0.8**	4.4±1.7	1.7±0.5
Cholesterol ^c (mmol/l)	4.2±0.9	3.5±0.6	6.7±1.0***	14.8±2.2***	3.4±0.4
NEFA ^c (mmol/l)	0.25±0.04	0.14±0.02**	0.18±0.01	0.29±0.05	0.21±0.07
Liver triglycerides (nmol/mg liver)	36.9±9.3	63.1±11.1**	33.8±5.1	174.6±40.1**	5.3±2.2**

^a Data are given in means ± S.D.

^b Blood parameters were analyzed from plasma unless stated otherwise.

^c log-transformed for ANOVA.

^d All groups were compared to untreated db/db; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

TABLE 2. Overall statistics of changes in gene expression profiles

Effect	Comparison vs	Number of genes ^a			
		untreated db/db	Increased	Decreased	Total
Diabetes ^{-1b}	db/+		155	182	337
PPAR γ	Rosi		158	142	300
PPAR α	Wy		362	258	620
LXR	T09		402	333	735

^a Number of genes changed at least ± 1.5 -fold and a $p < 0.01$.

^b The diabetes effect was given as reciprocal (db/+ vs db/db; diabetes⁻¹) in order to facilitate direct comparisons with normalizing effects of drugs.

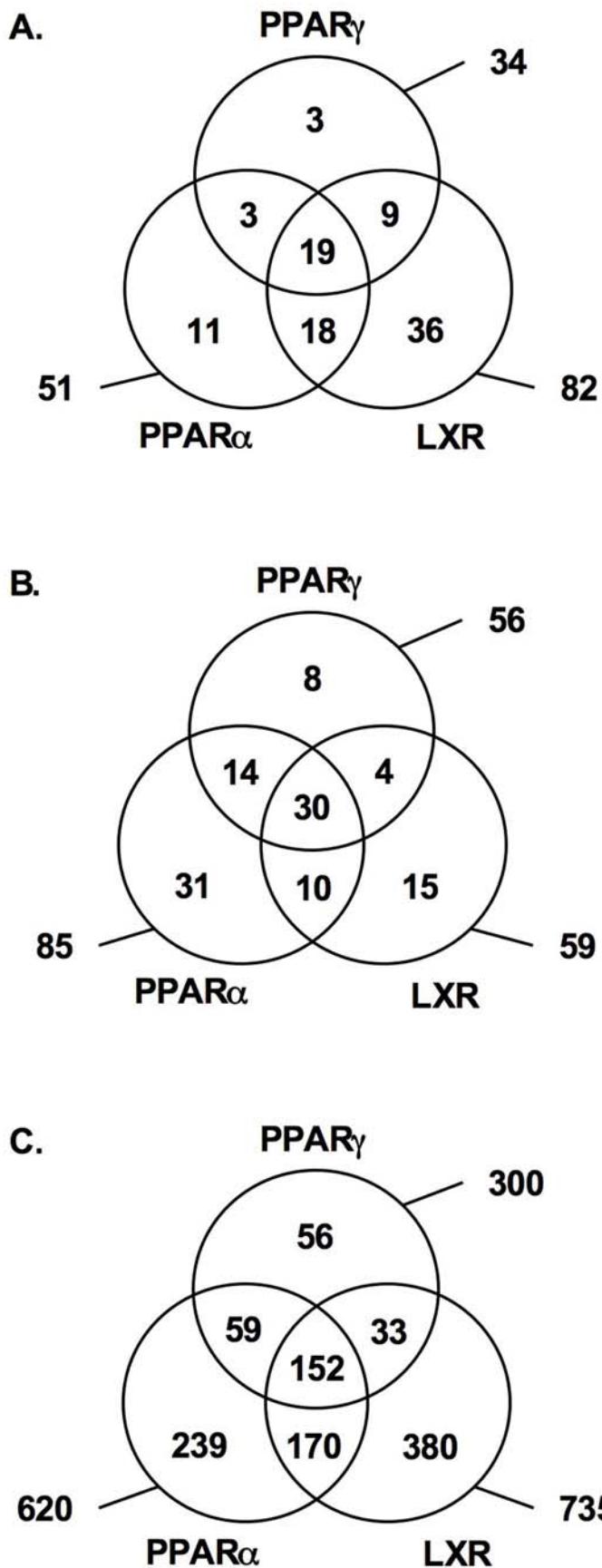


FIG. 1

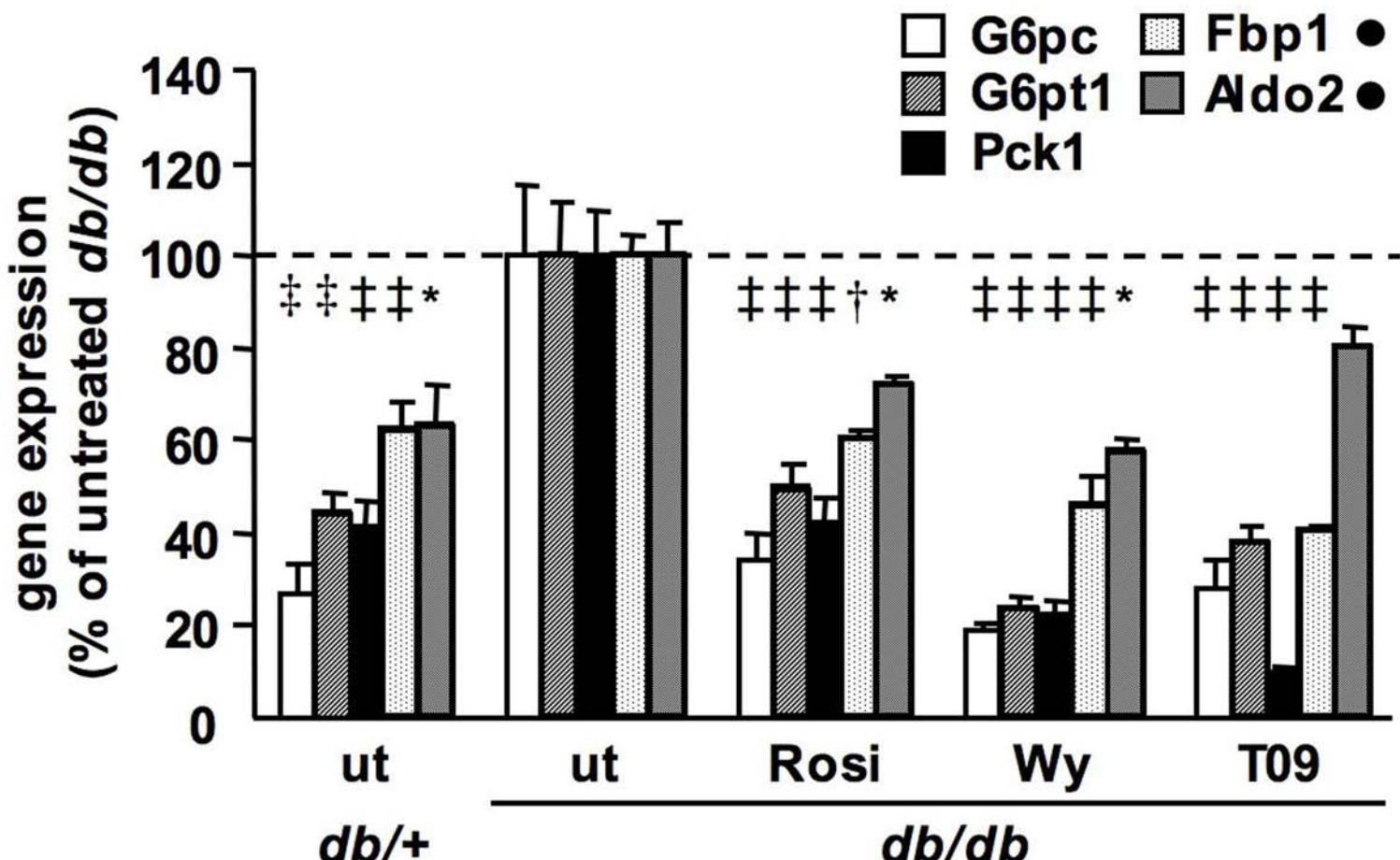
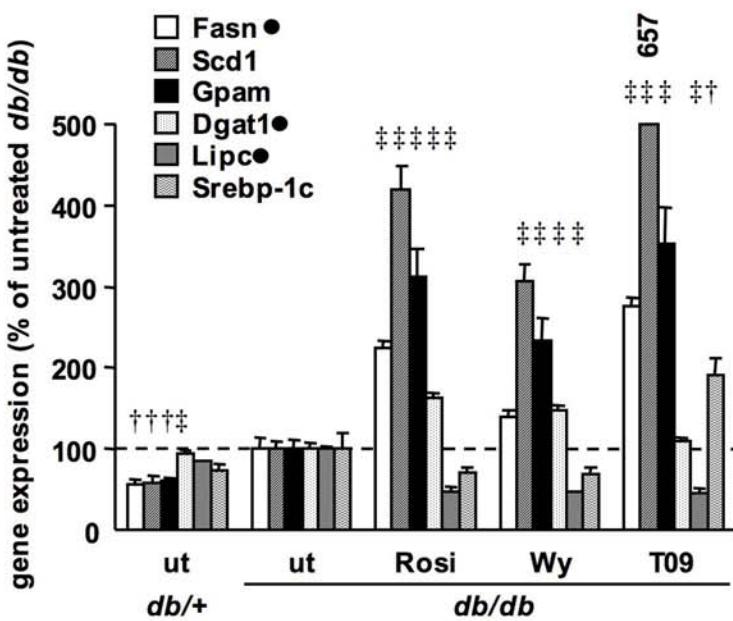
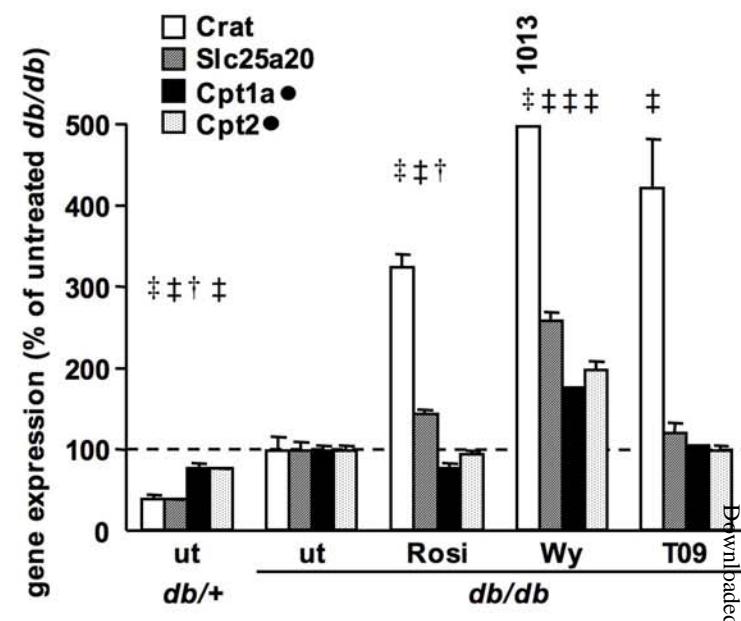


FIG. 2

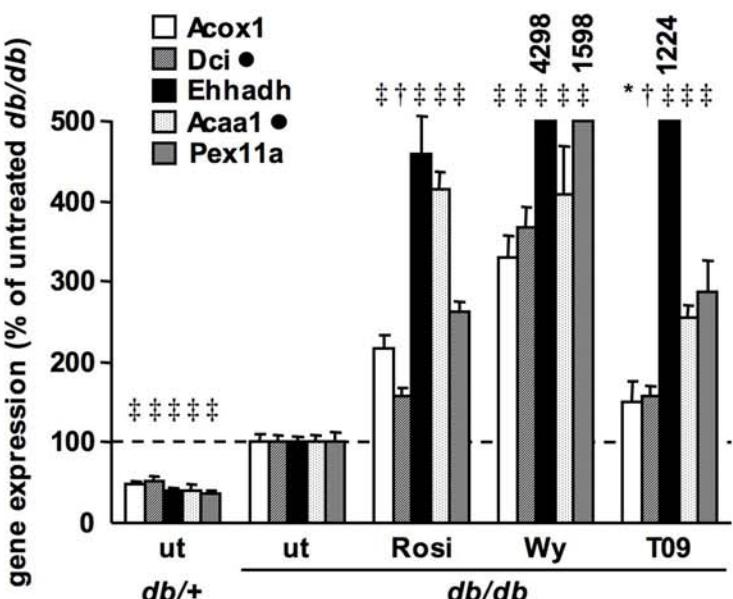
A



B



C



D

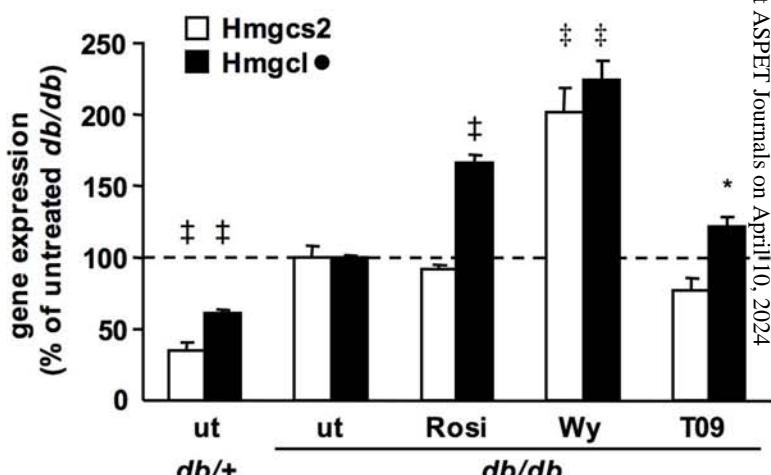


FIG. 3

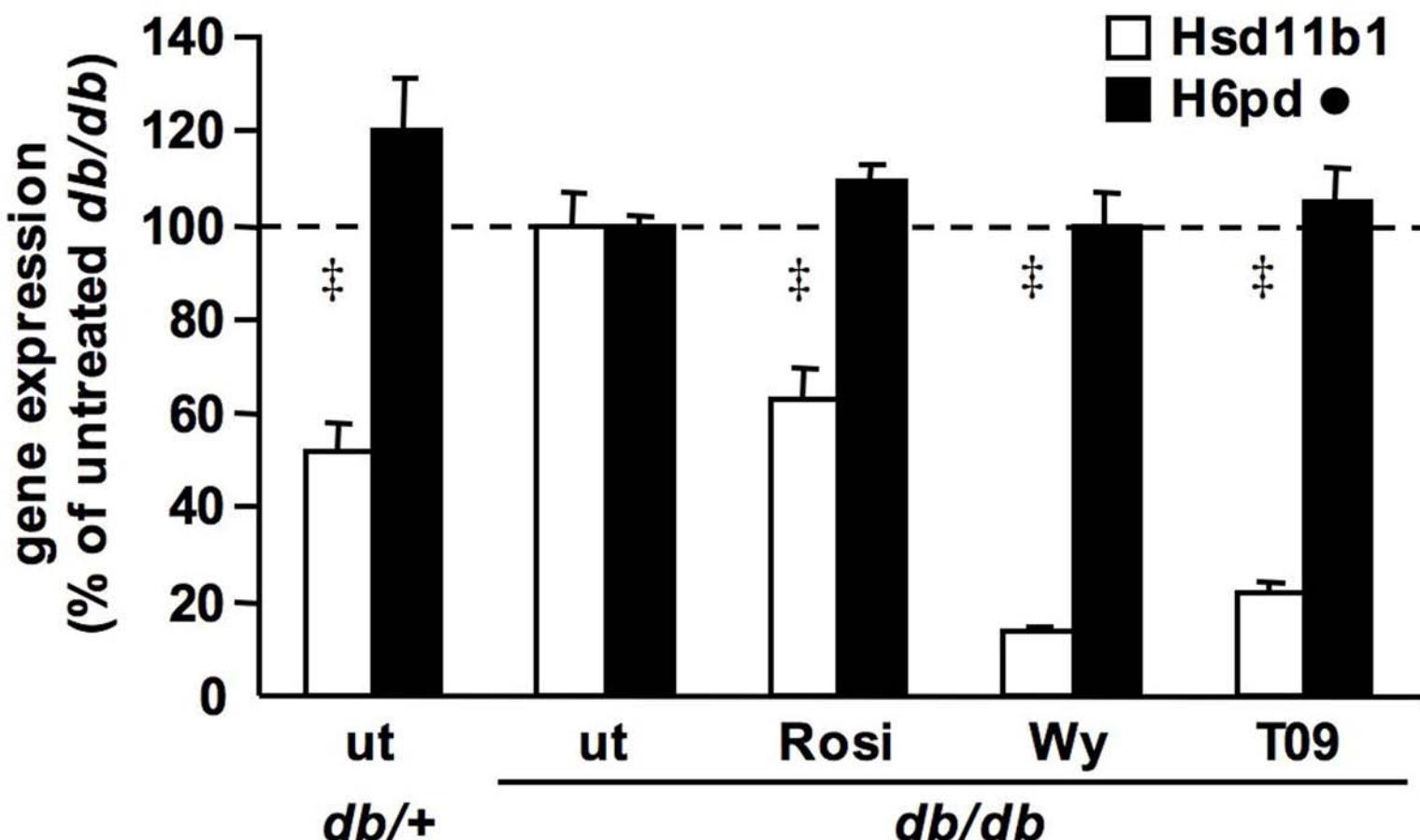


FIG. 4

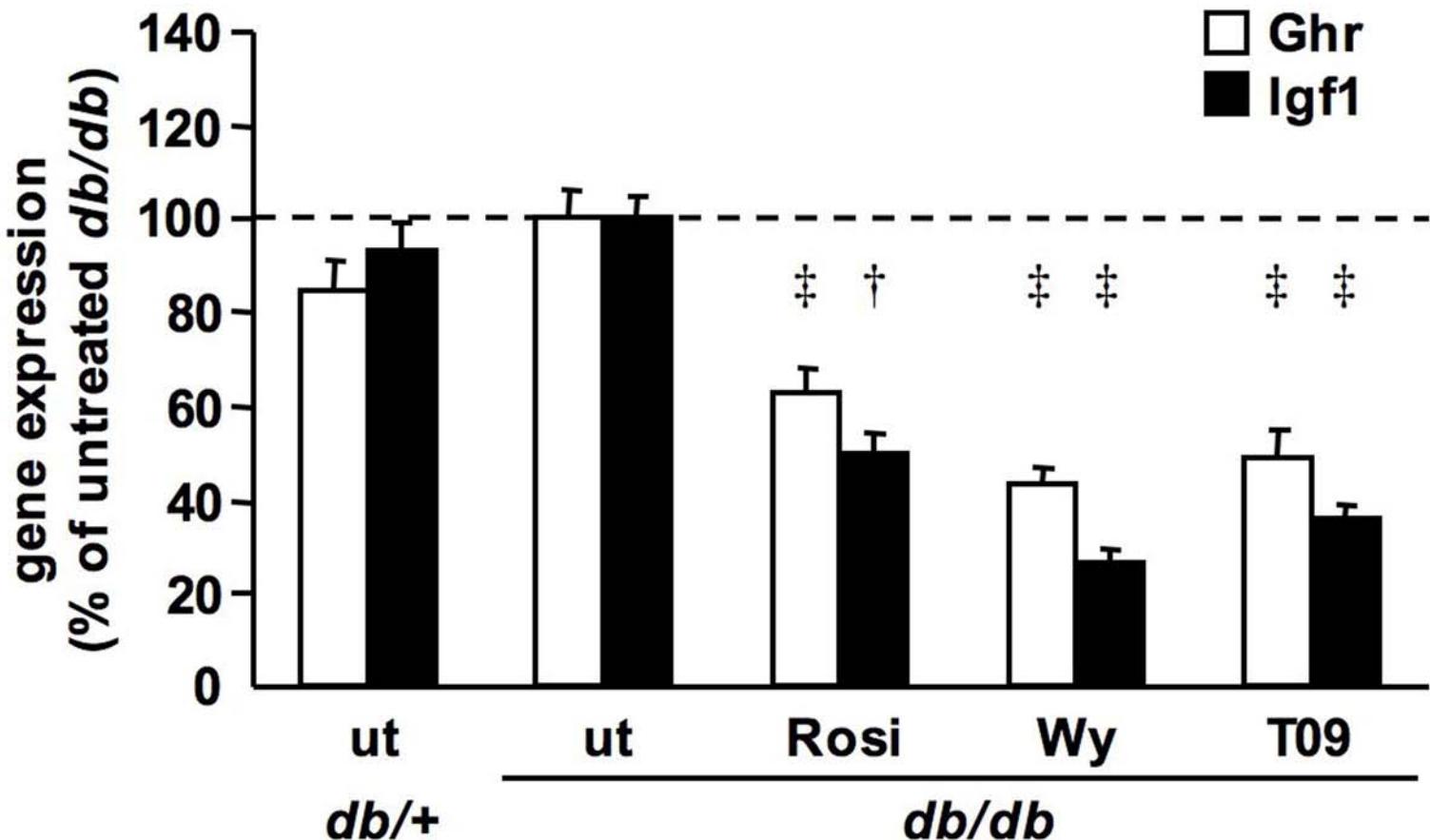


FIG. 5

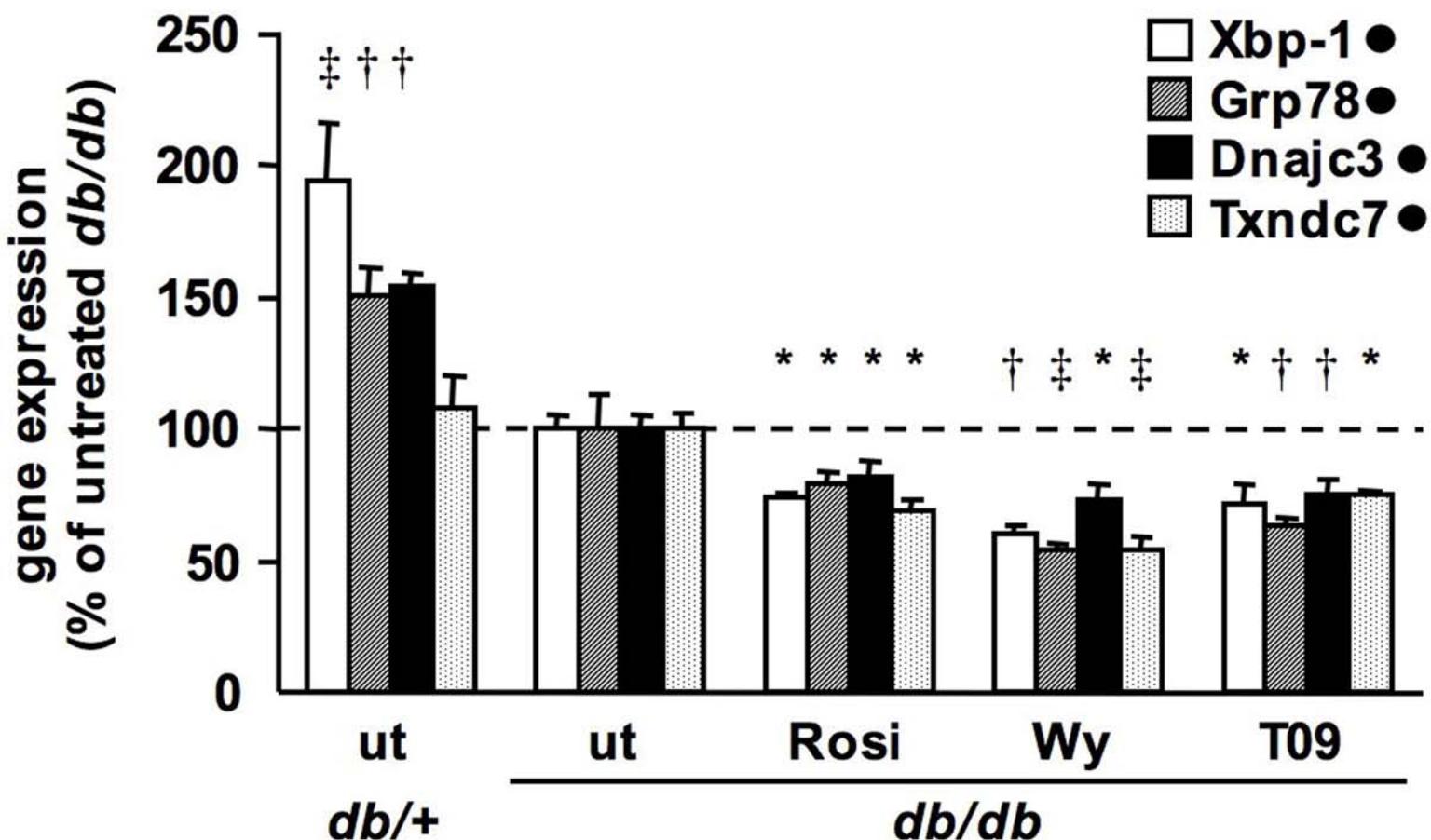


FIG. 6

Supplementary Figure 1A

List of genes regulated by all agonists in the same direction to db/+

affy ID#	genbank ID#	symbol	gene name	fold changes				estimate	
				B	W	T	+		
								db/db	
104617_at	AI042964	0610005	RIKEN cDNA 0610005C13 gene					-1,8 -2,5 -2,9 -1,5	795
104566_at	AI845009	Asl	argininosuccinate lyase					-2,3 -4,7 -3,1 -2,1	226
102373_at	M29961	Enpep	glutamyl aminopeptidase					-2,7 -3,5 -2,5 -2,0	260
95660_at	AI851815	Ethe1	RIKEN cDNA 0610025L15 gene					-1,7 -2,6 -1,9 -1,6	634
96918_at	AI790931	Fbp1	fructose bisphosphatase 1					-1,7 -2,2 -2,5 -1,6	3975
103333_at	U00445	G6pc	glucose-6-phosphatase, catalytic					-2,7 -2,9 -3,4 -2,9	2329
97430_at	AF080469	G6pt1	glucose-6-phosphatase, transport protein 1					-1,9 -3,8 -2,7 -1,6	901
96828_at	D89664	Gnmt	glycine N-methyltransferase					-1,5 -3,7 -3,3 -1,5	5650
97867_at	X83202	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1					-1,8 -5,9 -4,0 -1,6	1624
95611_at	AA726364	Lpl	lipoprotein lipase					3,8 17,111,2 2,3	31
160083_at	M63335	Lpl	lipoprotein lipase					2,6 11,1 7,2 1,9	18
101473_at	U86108	Nnmt	nicotinamide N-methyltransferase					-2,3 -7,0 -4,7 -1,7	1084
104343_f_at	AI845798	Pla2g12a	phospholipase A2, group XII					1,8 1,6 2,4 1,7	90
93926_at	M22957	Prlr	prolactin receptor					-2,0 -2,2 -2,7 -2,6	236
102816_at	X69832	Serpina3	serine (or cysteine) proteinase inhibitor, clade A, member					-2,8 -3,6 -2,7 -1,8	696
100341_g_at	U95132	Slc10a1	solute carrier family 10 (sodium/bile acid cotransporter					-1,8 -2,5 -4,2 -1,7	1414
104539_at	AF022894	Sult1b1	sulfotransferase family 1B, member 1					-1,6 -2,3 -2,4 -1,6	169
93662_s_at	AI386093	Zap70	zeta-chain (TCR) associated protein kinase					-2,5 -3,2 -3,6 -1,8	173
93661_at	U04379	Zap70	zeta-chain (TCR) associated protein kinase					-1,7 -2,3 -2,2 -1,8	78

B ... BRL vs untreated db/db ; W ... Wy vs untreated db/db ; T... T09 vs untreated db/db ; +... db/+ vs db/db

Fold change



Supplementary Figure 1B

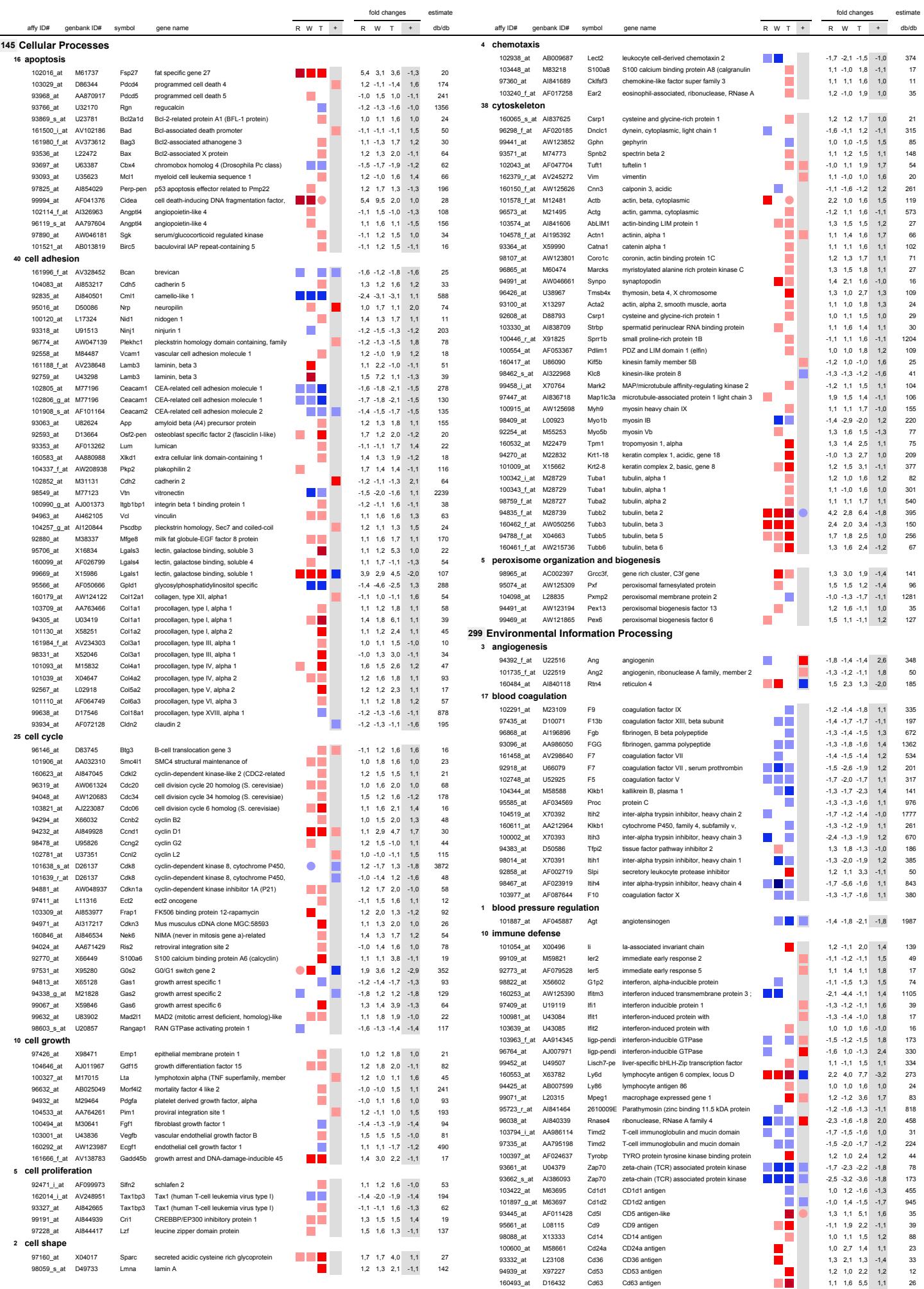
List of genes regulated by all agonists in the opposite direction to db/+

affy ID#	genbank ID#	symbol	gene name	fold changes				estimate	
				B	W	T	+		
99571_at	AW012588	Acaa1	3-ketoacyl-CoA thiolase B					2,5 2,8 2,0 -2,5	4993
102004_at	AI530403	Acaa1	3-ketoacyl-CoA thiolase B, acetyl-Coenzyme A					4,2 4,0 2,6 -2,7	836
98589_at	M93275	Adrp	adipose differentiation related protein					2,3 4,1 2,8 -1,6	812
99559_at	U14390	Aldh3a2	aldehyde dehydrogenase family 3, subfamily A2					2,0 4,1 2,1 -3,9	857
103703_f_at	AI647632	C730048	RIKEN cDNA C730048C13 gene					-1,8 -2,7 -2,7 1,7	312
103702_i_at	AI647632	C730048	RIKEN cDNA C730048C13 gene					-2,3 -3,6 -4,4 2,1	251
104424_at	X05475	C9	complement component 9					-2,4 -5,4 -2,0 1,8	1044
103646_at	X85983	Crat	carnitine acetyltransferase					3,0 6,6 2,8 -1,6	106
103581_at	Y14004	Cte1	cytosolic acyl-CoA thioesterase 1					2,2 20,0 4,6 -4,1	145
161345_f_at	AV141027	Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1					-2,1 -2,0 -2,6 12,8	65
92898_at	U36993	Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1					-2,6 -2,7 -3,6 12,8	108
98527_at	Z14050	Dci	dodecenoyl-Coenzyme A delta isomerase (3,2)					1,6 3,7 1,6 -1,9	449
101842_g_at	AW049716	Egfr	epidermal growth factor receptor					-3,1 -3,4 -3,1 2,5	219
101841_at	AW049716	Egfr	epidermal growth factor receptor					-4,2 -5,2 -4,7 2,5	136
101840_at	L06864	Egfr	epidermal growth factor receptor					-1,7 -1,8 -1,8 1,7	64
97316_at	AJ011864	Ehhadh	RIKEN cDNA 1300002P22 gene					4,0 22,1 6,9 -1,7	76
104325_at	AI461631	Falp	Fat cell-specific low molecular weight protein					2,1 2,1 1,7 -1,6	198
97820_at	AB027012	Galk1	galactokinase 1					2,5 1,6 2,0 -1,6	99
101659_at	M75886	Hsd3b2	hydroxysteroid dehydrogenase-2, delta<5>-3-beta					-5,5 -5,6 -7,3 5,4	115
99669_at	X15986	Lgals1	lectin, galactose binding, soluble 1					3,9 2,9 4,5 -2,0	107
104658_at	D17444	Lifr	leukemia inhibitory factor receptor					-3,5 -3,6 -4,0 3,4	330
104659_g_at	D17444	Lifr	leukemia inhibitory factor receptor					-3,5 -3,9 -4,1 3,2	197
160553_at	X63782	Ly6d	lymphocyte antigen 6 complex, locus D					2,2 4,0 7,7 -3,2	273
96258_at	AI843448	Mgst3	microsomal glutathione S-transferase 3					6,8 3,4 2,6 -2,1	98
101082_at	J02652	Mod1	malic enzyme, supernatant					3,7 5,8 3,7 -2,0	239
103660_at	AF093669	Pex11a	peroxisomal biogenesis factor 11a					2,7 5,5 2,2 -1,7	386
100927_at	U28960	Pltp	phospholipid transfer protein					8,2 7,6 4,6 -1,7	128
102013_at	AF030513	Rdh6	retinol dehydrogenase 6					2,7 2,1 1,9 -3,1	154
96038_at	AI840339	Rnase4	ribonuclease, RNase A family 4					-2,3 -1,6 -1,8 2,0	458
92242_at	U65403	Saa4	serum amyloid A 4					-2,1 -2,6 -3,3 1,6	227

B ... BRL vs untreated db/db ; W ... Wy vs untreated db/db ; T... T09 vs untreated db/db ; +... db/+ vs db/db

Fold change <-5,0 <-2,0 <-1,5 >1,5 >2,0 >5,0

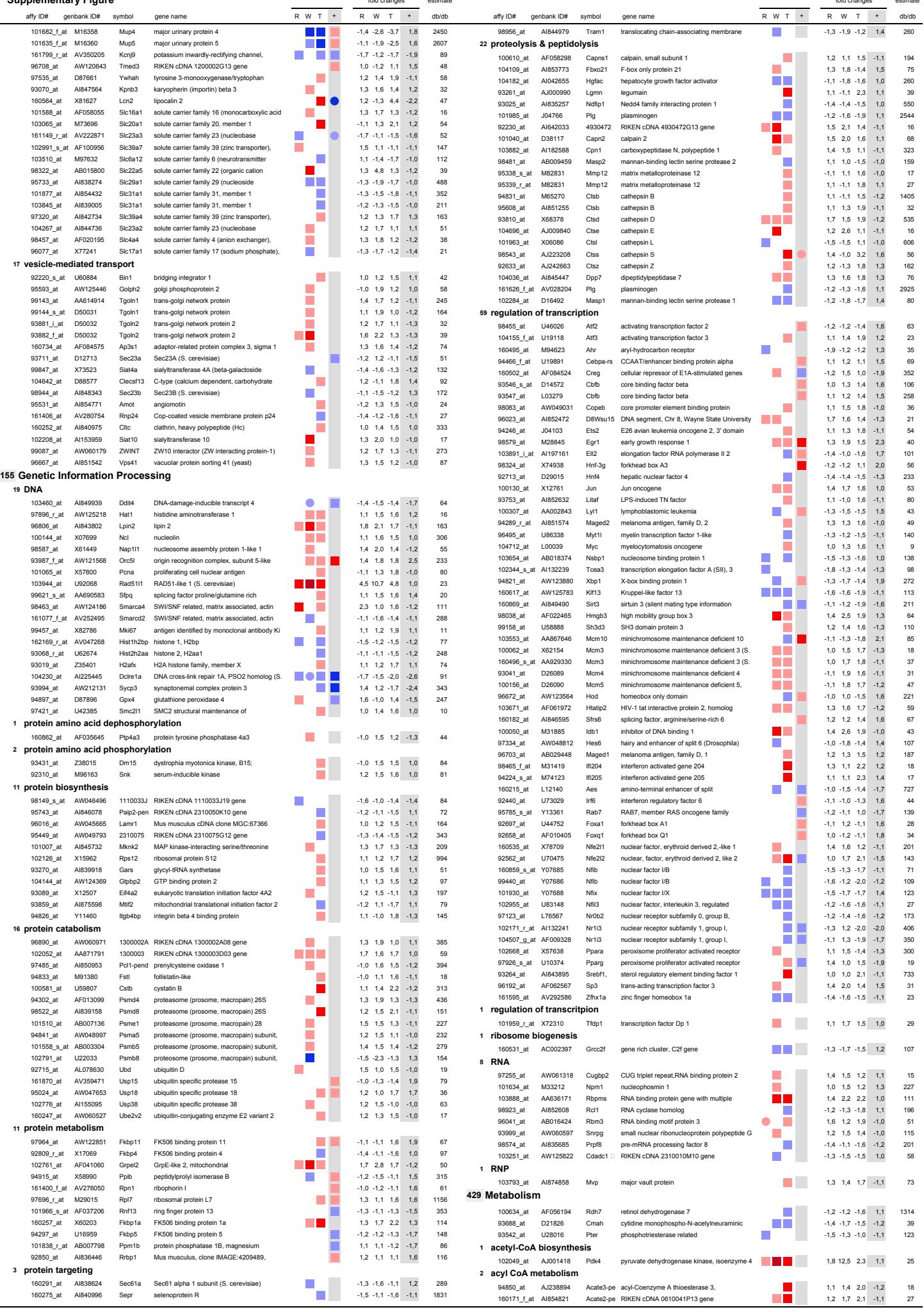
Supplementary Figure 3. Hepatic gene expression profiles of drug-treated *db/db* and untreated *db/+* compared to untreated *db/db*



Supplementary Figure

affy ID#	genbank ID#	symbol	gene name	fold changes				estimate				fold changes				estimate										
				R	W	T	+	R	W	T	+	db/db	affy ID#	genbank ID#	symbol	gene name	R	W	T	+	db/db					
103016_s_at	X68273	Cd68	CD68 antigen	-1.0	-1.0	1.8	1.2	-1.0	-1.0	1.8	1.2	34	99596_f_at	M13963	Gna12	guanine nucleotide binding protein, alpha	1.1	1.0	1.6	1.2	411					
98291_at	A1852553	Tmsb10	thymosin, beta 10	1.3	1.4	4.0	-1.2	68	99598_g_at	A1841629	Gna12	guanine nucleotide binding protein, alpha	1.1	1.1	1.6	1.1	290									
99862_at	AF025621	Ahsg	alpha-2-HS-glycoprotein	-1.6	-2.3	-1.6	1.3	-1.6	-2.3	-1.6	1.3	4606	101441_L_at	AFO31147	Itp5	inositol 1,4,5-triphosphate receptor 5	1.0	1.1	1.2	1.9	32					
161827_f_at	AV105307	Hpx	hemopexin	1.2	-1.5	2.0	1.6	1.2	-1.5	1.9	1.4	1078	160530_at	AW120976	Ghrm	growth hormone inducible transmembrane	1.0	1.5	1.0	1.0	102					
98116_at	U89889	Hpx	hemopexin	1.2	-1.5	1.9	1.4	1.2	-1.5	1.8	1.4	1823	99107_f_at	M31680	Ghr	growth hormone receptor	-1.7	-1.7	-2.1	-1.2	214					
102575_at	S38219	Orm3	orosomucoid 3	1.5	-1.8	-1.7	1.8	1.5	-1.8	-1.7	1.8	112	99108_s_at	U15012	Ghr	growth hormone receptor	-1.7	-2.3	-2.5	-1.0	496					
96787_at	AA808091	Serpina10	serine (or cysteine) peptidase inhibitor, clade	-1.3	-1.5	-1.1	1.4	-1.3	-1.5	-1.4	1.4	441	104658_at	D17444	Lifr	leukemia inhibitory factor receptor	-3.5	-3.6	-4.0	3.4	330					
106951_at	A1645694	Serpina12	serine (or cysteine) peptidase inhibitor, clade	-1.3	-1.5	-1.4	8.2	-1.3	-1.5	-1.4	8.2	47	104659_g_at	D17444	Lifr	leukemia inhibitory factor receptor	-3.5	-3.9	-4.1	3.2	197					
101565_f_at	M75720	Serpina1a	serine (or cysteine) peptidase inhibitor, clade	-1.3	-1.7	-1.8	1.1	-1.3	-1.7	-1.8	1.1	?	99142_at	A183495	B210021	membrane protegerin receptor alpha	1.9	1.9	2.1	1.0	96					
101572_f_at	M75721	Serpina1a	serine (or cysteine) peptidase inhibitor, clade	-1.4	-2.0	-2.0	1.3	-1.4	-2.0	-2.0	1.3	7060	104354_at	X06368	Csf1r	colony stimulating factor 1 receptor	1.2	-1.0	1.5	1.2	50					
101579_f_at	M25529	Serpina1b	serine (or cysteine) peptidase inhibitor, clade	-1.2	-1.7	-1.7	1.1	-1.2	-1.7	-1.7	1.1	8856	160674_at	A159694	Trif2	transferrin receptor 2	-1.2	-1.4	-1.6	-1.3	547					
100329_at	X00945	Serpina1d	serine (or cysteine) peptidase inhibitor, clade	-1.4	-2.2	-2.2	2.2	-1.4	-2.2	-2.2	2.2	7106	92847_s_at	M68631	M6pr	mannose-6-phosphate receptor, cation	1.2	1.6	1.3	1.1	53					
93109_f_at	M75718	Serpina1d	serine (or cysteine) peptidase inhibitor, clade	-1.2	-1.7	-1.8	1.1	-1.2	-1.7	-1.8	1.1	8718	94828_at	AF004927	Oprs1	opioid receptor, sigma 1	2.0	1.8	1.7	-1.3	428					
101574_f_at	M75717	Serpina1e	serine (or cysteine) peptidase inhibitor, clade	-1.3	-1.9	-2.0	1.3	-1.3	-1.9	-2.0	1.3	6961	97507_at	X67809	Lgals3bp	lectin, galactose binding, soluble, 3 binding	1.2	1.3	1.8	-1.0	84					
102707_f_at	X61597	Serpina3c	serine (or cysteine) peptidase inhibitor, clade	-1.7	-2.0	-2.2	1.1	-1.7	-2.0	-2.2	1.1	700	94801_at	A167332	Pgmc2	progesterone receptor membrane component	1.6	1.6	1.1	-1.1	35					
92583_at	D00725	Serpina3k	serine (or cysteine) peptidase inhibitor, clade	-1.9	-3.0	-3.3	-1.1	-1.9	-3.0	-3.3	-1.1	64	96104_at	AI047107	3732413	RIKEN cDNA A73c24131H gene	1.3	1.6	3.2	1.2	30					
102816_at	X69832	Serpina3	serine (or cysteine) peptidase inhibitor, clade	-2.8	-3.6	-2.7	-1.8	-2.8	-3.6	-2.7	-1.8	696	96534_at	AA408896	Vldlr	very low density lipoprotein receptor	1.3	2.0	1.1	-1.0	11					
104374_at	M64086	Serpina3n	serine (or cysteine) peptidase inhibitor, clade	-1.6	-1.5	-1.5	1.0	-1.6	-1.5	-1.5	-1.0	806	55 signal transduction													
96227_at	X70553	Serpina12	serine (or cysteine) peptidase inhibitor, clade	-1.3	-1.1	-1.8	-1.6	-1.3	-1.1	-1.8	-1.6	859	99108_at	AF071315	Cops6	COP9 (constitutive photomorphogenic)	1.2	1.8	1.2	-1.3	448					
96609_at	U25844	Serpina6a	serine (or cysteine) peptidase inhibitor, clade	-1.3	1.3	4.3	1.7	-1.3	1.3	4.3	1.7	20	96592_at	U50413	Pik3r1	phosphatidylinositol 3-kinase, regulatory	-1.1	1.5	1.2	-1.4	54					
100966_at	U07425	Serpind1	serine (or cysteine) peptidase inhibitor, clade	-1.6	-1.5	-1.8	1.1	-1.6	-1.5	-1.8	1.1	1025	102338_at	AF031147	Pde6a	phosphodiesterase 9A	-1.2	-1.6	-1.3	-1.1	34					
101928_at	Z36774	Serpinf1	serine (or cysteine) peptidase inhibitor, clade	-1.5	-2.3	-1.9	1.3	-1.5	-2.3	-1.9	1.3	599	100555_at	AI846152	Dscr1	Down syndrome critical region homolog 1	-1.3	-1.7	-1.1	-2.4	131					
94817_at	X60676	Serpinf1	serine (or cysteine) peptidase inhibitor, clade	-1.1	-1.1	-1.7	1.1	-1.1	-1.1	-1.7	1.1	32	161610_at	AV34986	Ndr2	N-myco downstream regulated 2	-1.4	-1.8	-2.7	1.5	419					
97444_at	A1844520	If30	interferon gamma inducible protein 30	1.2	1.2	2.3	1.0	1.2	1.2	2.3	1.0	39	96088_at	AB03921	Ndr2	N-myco downstream regulated 2	-1.3	-1.9	-2.4	1.1	1165					
104407_at	L25274	Alcan	activated leukocyte cell adhesion molecule	-1.1	-1.0	1.2	1.8	-1.1	-1.0	1.2	1.8	17	104268_at	X51975	Ilra	interleukin 6 receptor	-1.5	-1.9	-1.9	-1.0	92					
93454_at	AF0181789	C1q1r	complement component 1, q subcomponent	1.0	1.0	1.6	1.1	1.0	1.0	1.6	1.1	31	102321_at	M93422	Adcy6	adenylyl cyclase 6	1.3	1.5	1.2	-1.0	54					
102961_at	A111838	Hrg	histidine-rich glycoprotein	-1.7	-2.7	-3.1	-1.0	-1.7	-2.7	-3.1	-1.0	1043	104412_at	AI153412	Gnat1	guanine nucleotide binding protein, alpha	1.4	1.8	1.1	-1.1	27					
100882_at	AF003525	Defb1	defensin beta 1	1.2	2.2	2.2	-1.1	1.2	2.2	2.2	-1.1	19	161609_at	AV349152	Rgs16	regulator of G-protein signaling 16	1.0	-2.8	-2.4	-2.1	83					
92852_at	M18194	F1n1	fibronectin 1	-1.5	-1.3	-1.4	-1.1	-1.5	-1.3	-1.4	-1.1	408	93783_at	M27347	Ela1	elastase 1, pancreatic	1.2	1.0	1.1	2.4	120					
104388_at	U49513	Syc9	chemokine (C-C motif) ligand 9	1.8	1.2	2.6	1.5	1.8	1.2	2.6	1.5	127	97227_at	M63659	Gna12	guanine nucleotide binding protein, alpha 12	-1.1	1.0	-1.3	-1.8	43					
99671_at	X04673	Adipn	adipon	13.5	-1.0	-1.1	1.1	13.5	-1.0	-1.1	1.1	34	92647_at	U35141	Rbpb4	retinoblastoma binding protein 4	-1.1	-1.1	-1.1	1.8	52					
104424_at	X05475	C9	complement component 9	-2.4	-5.4	-2.0	-1.8	-2.4	-5.4	-2.0	-1.8	1044	93559_at	D90374	Apex1	apurinic/aprimidinic endonuclease 1	1.1	1.5	1.4	1.1	105					
101853_f_at	M12660	Cfh	complement component 1, q subcomponent	-1.3	-1.5	-1.7	1.2	-1.3	-1.5	-1.7	1.2	195	97458_at	AI845935	Gnb1	guanine nucleotide binding protein, beta 1	1.5	1.0	1.4	1.2	114					
94743_at	M29009	Cfh	complement component factor h	-1.6	-2.1	-2.1	1.2	-1.6	-2.1	-2.1	1.2	300	93771_at	X84215	Gjb1	gap junction membrane channel protein beta	-1.4	-1.3	-1.7	-1.1	1025					
94994_at	M29007	Cfh	complement component factor h	-1.8	-1.8	-1.9	-1.2	-1.8	-1.8	-1.9	-1.2	62	102366_at	AA718169	Retn	resistin	-3.7	-1.2	-1.1	-1.0	47					
103458_at	M35525	Hc	hemolytic complement	-1.5	-1.9	-1.4	2.2	-1.5	-1.9	-1.4	2.2	535	93212_at	Z97207	AW74231	butyrate-induced transcript 1	1.0	2.1	1.0	-1.8	81					
101468_at	X120905	Pfc	properdin factor, complement	1.2	1.0	1.8	-1.0	1.2	1.0	1.8	-1.0	23	160320_at	U58883	Sorbs1	sorbin and SH3 domain containing 1	1.6	1.8	1.4	-1.3	114					
98562_at	X58861	C1q1a	complement component 1, q subcomponent	1.2	-1.1	2.1	1.2	1.2	-1.1	2.1	1.2	80	97803_at	U38196	Mpp1	membrane protein, palmitoylated	1.2	2.1	1.9	1.0	116					
96020_at	M22631	C1qb	complement component 1, q subcomponent	1.5	-1.0	3.1	1.2	1.5	-1.0	3.1	1.2	61	93747_at	AW122599	Hscd12	butyrate-induced transcript 1	1.1	2.2	1.2	-1.0	644					
92223_at	X62629	C1q1g	complement component 1, q subcomponent	1.1	-1.0	1.9	1.0	1.1	-1.0	1.9	1.0	140	97909_at	AI838090	Stmn1	stathmin 1/oncoprotein 18	1.1	1.9	2.7	-1.2	54					
103673_at	M57891	C2	complement component 2 (within H-2S)	-1.1	-1.4	-1.1	1.7	-1.1	-1.4	-1.1	1.7	109	103020_s_at	AI317205	Map3k1	mitogen activated protein kinase kinase kinase	1.2	1.1	1.4	1.6	26					
102799_at	M17122	C4bp	complement component 4 binding protein	-1.5	-2.8	-1.7	-1.2	-1.5	-2.8	-1.7	-1.2	927	102195_at	U88984	Map4k4	mitogen-activated protein kinase kinase kinase	1.3	1.4	1.4	-1.0	17					
103033_at	X06454	C4	complement component 4 (within H-2S)	1.1	-1.8	-1.5	1.1	1.1	-1.8	-1.5	1.1	1675	93285_at	AI845584	Dusp6	diaphanous-related protein 6 specificity phosphatase 6	1.2	1.2	2.0	-1.4	57					
99927_at	U47810	Cfi	complement component factor i	-1.3	-1.5	-1.3	1.2	-1.3	-1.5	-1.3	1.2	1132	161257_r_at	AV321519	Snx17	sorting nexin 17	-1.2	-1.3	-1.5	2.4	61					
101474_at	U09010	Mbl1	mannose binding lectin, liver (A)	1.1	-1.0	3.7	1.0	1.1	-1.0	3.7	1.0	306	96927_at	AI85048	Rab6	RAB6, member RAS oncogene family	1.0	-1.1	-1.1	-1.1	142					
97427_at	U09016	Mbl2	mannose binding lectin, serum (C)	1.2	-1.0	3.7	1.0	1.2	-1.0	3.7	1.0	306	95516_at	AB027290	Rab9	RAB9, member RAS oncogene family	1.1	2.3	1.3	-1.1	190					
100112_at	L12030	Cxcl12	chemokine (C-X-C motif) ligand 12	-1.5	-1.8	-1.8	-1.1	-1.5	-1.8	-1.8	-1.1	212	95516_at	AB027290	Rab9	RAB9, member RAS oncogene family	1.2	1.8	1.2	1.0	24					
160511_at	L12029	Cxcl12	chemokine (C-X-C motif) ligand 12	-1.1	-1.3	-1.5	-1.1	-1.1	-1.3	-1.5	-1.1	64	95547_at	AB98921	Rhod	ras homolog gene family, member D	1.3	1.3	1.6	-1.1	98					
162234_f_at	AV19913	Cxcl12	chemokine (C-X-C motif) ligand 12	-1.2	-1.4	-1.6	-1.1	-1.2	-1.4	-1.6	-1.1	60	94362_at	AI843682	Nras	neuroblastoma ras oncogene	1.1	1.5	1.6	1.6	54					
97519_at	X13986	Spp1	secreted phosphoprotein 1	-1.1	-1.6	3.6	1.2	-1.1	-1.6	3.6	1.2	129	101030_at	X99963	Arh	ras homolog gene family, member AB	1.0	1.4	1.8	1.2	58					
92866_at	X52643	H2-Aa	histocompatibility 2, class II antigen A, alpha	1.1	-1.1	1.9	1.2	1.1	-1.1	1.9	1.2	95	96056_at	X80638	Arhc	ras homolog gene family, member C	1.1	1.2	1.6	1.0	56					
97541_f_at	X02246	H2-D1	histocompatibility 2, D region locus 1	1.2	-1.2	1.2	1.5	1.2	-1.2	1.2	1.5															

Supplementary Figure



Supplementary Figure

affy ID#	genbank ID#	symbol	gene name	fold changes			estimate			affy ID#	genbank ID#	symbol	gene name	fold changes			estimate				
				R	W	T	+	R	W	T	+	db/db		R	W	T	+	db/db			
1 amino acid activation																					
100136_at	M32017	Lamp2	lysosomal membrane glycoprotein 2	[blue]	[grey]	-1.3	-1.3	-1.7	-1.1	315	95066_at	U67611	Tald1	transaldolase 1	[red]	[red]	1.7	1.3	1.3	-1.4	327
1 amino acid catabolism																					
98966_at	L42998	Dbt	dihydroliopamide branched chain	[blue]	[grey]	-1.0	-1.2	-1.6	1.1	75	101294_g_at	Z84471	G6pd2	glucose-6-phosphate dehydrogenase 2	[red]	[red]	1.5	1.2	2.1	1.1	21
53 amino acid metabolism																					
93625_at	AA986718	Agtx	alanine-glyoxylate aminotransferase	[blue]	[grey]	-1.4	-1.6	-3.7	1.3	274	101964_at	U05089	Tkt	transketolase	[blue]	[red]	1.5	1.3	1.5	-1.5	295
104286_at	AA795541	Slc38a4	solute carrier family 38, member 4	[blue]	[blue]	-1.4	-1.1	-1.5	-1.0	1991	95420_at	AW120625	Pgd	phosphoglucomate dehydrogenase	[red]	[red]	1.3	1.4	2.0	-1.1	119
104007_at	AA986782	Slc25a15	solute carrier family 25 (mitochondrial carrier)	[blue]	[blue]	-2.1	-2.2	-2.3	-1.1	540	97260_at	AW121489	Agrp1-p	cyclic AMP phosphoprotein	[blue]	[blue]	-1.0	1.8	1.6	-1.1	36
94433_at	AW060684	Slc38a2	solute carrier family 38, member 2	[blue]	[blue]	-1.6	-1.5	-1.7	-1.0	152	103983_at	AI046345	Adh4	alcohol dehydrogenase 4 (class II), pi	[blue]	[blue]	-2.1	-2.2	-2.1	-1.2	227
92736_at	L03290	Slc7a2	solute carrier family 7 (cationic amino acid)	[blue]	[blue]	-2.0	-1.3	-1.1	0.2	161401_f_at	AV276715	Aldh3a2	aldehyde dehydrogenase family 3, subfamily	[red]	[red]	-1.9	-1.9	-2.0	-1.4	75	
100323_at	Z23077	Amd1	S-adenosylmethionine decarboxylase 1	[blue]	[red]	-1.5	-1.2	1.2	1.6	98	99559_at	U14390	Aldh3a2	aldehyde dehydrogenase family 3, subfamily	[red]	[red]	1.5	2.5	1.4	-2.4	857
101489_at	D12780	Amd1	S-adenosylmethionine decarboxylase 1	[blue]	[red]	1.1	1.4	2.4	1.0	103	97449_at	AI835461	Aldh7a1	aldehyde dehydrogenase family 7, member	[red]	[red]	2.0	4.1	2.1	-3.9	1293
96657_at	L10244	Sat	spermidine/spermine N1-acetyl transferase 1	[blue]	[red]	1.1	-2.6	-1.5	1.2	2018	100977_at	AA691492	Pdk1	pyruvate dehydrogenase kinase, isoenzyme 1	[red]	[red]	-1.1	-1.1	-1.5	-1.5	163
96346_at	AI854020	Cdo1	cysteine dioxygenase 1, cytosolic	[blue]	[red]	-1.3	-1.1	-2.7	-1.4	453	101471_at	D63764	Pkr	pyruvate kinase liver and red blood cell	[blue]	[blue]	-1.9	-3.7	-1.4	-3.2	442
103452_at	AA675075	Prodh2	proline dehydrogenase (oxidase) 2	[blue]	[red]	1.3	1.3	1.6	0.0	60	101472_s_at	AI195164	Pkr	pyruvate kinase liver and red blood cell	[blue]	[blue]	-1.5	-2.3	-1.2	-1.9	233
97385_at	JL242909	Nagl	N-acetylgalactosamine kinase	[red]	[red]	-1.1	1.7	1.2	-1.0	98	95060_at	AF050804	Slc16a7	solute carrier family 16 (monocarboxylic acid	[red]	[red]	-1.4	-1.1	-1.7	-1.0	635
99993_at	U77083	Anpep	alanyl (membrane) aminopeptidase	[red]	[blue]	-1.4	-1.4	-1.9	-1.2	900	100042_at	AS187921	Hagh	hydroxyacyl glutathione hydrolase	[red]	[red]	3.7	5.8	3.7	-2.0	239
93015_at	X65021	Gst3a	glutathione S-transferase, alpha 3	[blue]	[blue]	-1.0	-1.7	-1.0	1.3	154	101082_at	J02652	Mod1	malic enzyme, supernatant	[red]	[red]	1.9	1.0	-1.2	-1.5	253
96085_at	L06047	Gst4	glutathione S-transferase, alpha 4	[blue]	[blue]	1.4	1.6	2.4	-1.0	782	93308_s_at	M97957	Pcx	pyruvate carboxylase	[red]	[red]	-1.3	-1.9	-1.5	-1.4	307
102094_f_at	AI841270	Gstm1	glutathione S-transferase, mu 1	[red]	[red]	1.4	1.7	3.1	-1.1	4522	103416_at	AI844810	Mapk8	mitogen-activated protein kinase 6	[blue]	[blue]	1.5	1.5	2.0	1.5	76
93543_f_at	J03952	Gstm1	glutathione S-transferase, mu 1	[red]	[red]	1.2	-1.4	4.8	-1.5	83	94481_at	AI851321	Upgr2	UDP-glucose pyrophosphorylase 2	[blue]	[blue]	-1.7	-2.1	-1.1	-1.3	167
4 catecholamine metabolism																					
103909_at	J04696	Gstm2	glutathione S-transferase, mu 2	[red]	[red]	1.4	2.3	15.6	-1.2	309	103597_at	X63349	Dct	dopachrome tautomerase	[blue]	[blue]	-1.3	-2.0	-2.5	-1.4	146
97681_f_at	J03953	Gstm3	glutathione S-transferase, mu 3	[red]	[red]	6.8	3.4	2.6	-2.1	98	98920_g_at	AA867773	Bip1-pend	RIKEN cDNA 2410018G23 gene	[blue]	[blue]	-1.3	-1.2	-1.5	-1.3	90
97682_r_at	J03953	Gstm3	glutathione S-transferase, mu 3	[red]	[blue]	-1.1	-1.6	-1.7	-1.2	393	103421_at	D50464	Sdfr2	stromal cell derived factor receptor 2	[red]	[red]	-1.2	1.3	1.6	1.4	32
96258_at	AI843448	Mgst3	microsomal glutathione S-transferase 3	[red]	[blue]	-1.4	-1.9	-2.2	-1.3	913	93749_at	AI840845	Maoa	monoamine oxidase A	[red]	[red]	1.0	1.3	1.7	-1.4	58
12 cofactors & vitamins																					
104086_at	AI787269	Dmgdh	dimethylglycine dehydrogenase precursor	[blue]	[blue]	-1.5	-3.7	-3.3	-1.5	5650	94241_at	AI837229	Coasy-pe	Coenzyme A synthase	[red]	[red]	1.9	1.6	1.3	-1.1	130
96828_at	D89664	Gmrt	glycine N-methyltransferase	[blue]	[blue]	-1.4	-2.4	-2.4	-1.2	422	93736_at	AF090686	Tcn2	transcobalamin 2	[blue]	[blue]	1.1	-1.1	1.1	-1.7	316
101844_at	U94700	Pipox	picopelic acid oxidase	[blue]	[blue]	-1.5	-3.4	-3.6	-1.6	465	102313_at	L09737	Gch	GTP cyclohydrolase 1	[blue]	[blue]	-1.4	-1.7	-1.2	-1.0	174
96763_at	AI83995	Sard	sarcosine dehydrogenase	[blue]	[blue]	-1.3	-3.5	-6.9	-1.1	638	160850_at	U32197	Fpgs	folyl polyglutamyl synthetase	[red]	[red]	-1.0	1.1	-1.8	-1.0	407
92833_at	L07645	Hal	histidine ammonia lyase	[blue]	[blue]	1.1	-1.1	1.8	-1.2	95	99009_at	Z49204	Nit	nicotinamide nucleotide transhydrogenase	[red]	[red]	1.0	1.7	-1.2	-1.3	163
97424_at	AW049647	Arl6ip5	ARL6-iposylation factor-like 6 interacting	[blue]	[blue]	-1.2	-1.5	-1.9	-1.1	102	101473_at	U61806	Nnm1	nicotinamide N-methyltransferase	[blue]	[blue]	-2.3	-7.0	-4.7	-1.7	1084
96295_at	AW120230	Pst1	phosphoserine aminotransferase 1	[blue]	[blue]	1.1	1.8	1.5	-1.0	15	102013_at	AF030513	Rdh6	retinol dehydrogenase 6	[red]	[red]	2.7	2.1	1.9	-3.1	154
104539_at	AI72264	Sult1b1	sulfotransferease family 1B, member 1	[blue]	[blue]	1.1	1.9	-1.2	-1.1	333	96678_at	AW206761	Dhrs4	dehydrogenase/reductase (SDR family)	[red]	[red]	1.1	2.3	1.0	-1.7	427
92492_at	AB020203	Ak31	adenylyl kinase 3-alpha-like	[blue]	[blue]	-1.2	-1.5	-2.3	-1.9	109	102313_at	U32197	Cdc2a	cytchrome P450, family 26, subfamily	[red]	[red]	1.0	1.1	-1.2	-1.6	172
93575_at	U43285	Sps2	seleophosphate synthetase 2	[blue]	[blue]	-1.1	-1.7	-1.9	-1.0	5563	104603_at	X98056	Gst2	glutathione S-transferase, theta 1	[red]	[red]	1.8	1.1	-1.3	-1.3	750
92589_at	AI865645	Pspk	phosphoserine phosphatase	[red]	[blue]	-1.3	-1.6	-1.7	-1.0	507	104603_at	X98056	Gst2	glutathione S-transferase, theta 2	[red]	[red]	2.0	3.3	1.7	-1.2	293
99184_at	AW120896	Csd4	cysteine sulfide acid decarboxylase	[red]	[blue]	-1.2	-1.9	-2.1	-1.3	4843	98320_at	Y12657	Cyp26a1	cytochrome P450, family 26, subfamily a,	[red]	[red]	-1.8	-3.6	-4.3	-3.2	168
93827_at	U24493	Tdo2	tryptophan 2,3-dioxygenase	[red]	[blue]	-1.4	-1.2	-2.3	-1.0	518	102649_s_at	D64162	Ract1c	retinol acid early transcript gamma	[blue]	[blue]	1.3	2.0	-2.1	-5.0	641
99269_g_at	AI194855	Tdo2	tryptophan 2,3-dioxygenase	[red]	[blue]	-1.5	-1.1	-2.9	-1.3	2720	104716_at	U63637	Rbp1	retinol binding protein 1, cellular	[red]	[red]	1.4	1.3	2.4	2.2	38
98562_at	U58988	Hgd	homogentisate 1,2-dioxygenase	[blue]	[blue]	-1.3	-2.1	-1.5	-1.5	1399	93780_at	AW060827	Them2	thioesterase superfamily member 2	[red]	[red]	1.9	1.6	1.3	-1.4	130
104568_at	AI845009	Asl	argininosuccinate lyase	[blue]	[blue]	-2.3	-4.7	-3.1	-2.1	226	100596_at	MS3023	Selenbp1	seleum binding protein 1	[red]	[red]	1.2	-1.1	-1.4	3.0	828
92848_at	X64837	Oat1	ornithine aminotransferase	[blue]	[blue]	-1.5	-5.4	-2.6	-2.6	2268	162030_at	AV368672	Selenbp1	seleum binding protein 1	[blue]	[blue]	-1.5	-1.0	-1.5	-1.7	47
101408_at	AF010499	Gamt	guanidinoacetate methyltransferase	[blue]	[blue]	-1.3	-1.7	-2.4	-2.1	466	101587_at	U89491	Ephx1	epoxide hydrolase 1, microsomal	[blue]	[blue]	-1.7	1.5	1.3	-2.4	1430
94414_at	X70792	Otc	ornithine carbamoyltransferase	[blue]	[blue]	-1.1	-2.9	-2.4	-2.1	1098	93051_at	Z37107	Ephx2	epoxide hydrolase 2, cytoplasmic	[red]	[red]	1.9	2.6	1.0	-1.3	279
104153_at	AW047743	Ivd	isovaleryl coenzyme A dehydrogenase	[blue]	[blue]	1.3	1.1	-1.5	1.1	153	93021_at	AA790008	Drip1	detourinatal pallidolysis atrophy	[blue]	[blue]	1.1	-1.5	-1.5	-2.7	53
73 carbohydrate metabolism																					
102807_at	AW048054	9230112	RIKEN cDNA 9230112O05 gene	[red]	[blue]	1.1	1.7	1.5	-1.4	145	95660_at	AI851815	Ethe1	ethylmalonic encephalopathy 1	[blue]	[blue]	-1.1	-1.1	-1.4	-1.9	549
100565_at	AW123936	Gnplda	glucosamine-6-phosphate deaminase 1	[blue]	[blue]	-1.6	-1.6	-1.4	-1.5	64	160637_at	AW606325	Mocs2	molybdenum cofactor synthesis 2	[blue]	[blue]	-1.6	-2.0	-2.1	-1.6	328
97924_at	JL12236	Uae1	UDP-N-acetylglucosamine-2-epimerase/N-ac-	[blue]	[blue]	-1.2	-1.3	-1.6	1.1	366	160537_at	AF026073	Sult1	N-sulfotransferase	[blue]	[blue]	-1.6	-1.7	-1.8	1.9	32
96115_at	U28168	Dp1	deleted in polyosis 1	[blue]	[blue]	1.5	1.5	2.1	1.2	107	94797_at	AI120514	Sltc2a1	solute carrier family 26 (sulfate transporter),	[blue]	[blue]	-1.2	-2.0	-4.1	-1.2	192
103781_at	U78632	Stx4a	syntaxis 4A (placental)	[red]	[blue]	1.5	1.4	1.5	1.5	169	100691_at	U11333	E531	esterase 31	[red]	[red]	-1.1	-1.1	-1.2	-1.7	335
96870_at	AI83740	Aco2	aconitase 2, mitochondrial	[red]	[blue]	1.4	-1.0	1.6	1.0	443	94540_at	AI597772	Cyp2d6	cytochrome P450, family 26, subfamily d,	[red]	[red]	-1.4	-1.0	1.4	4.0	1853
160207_at	AW121639	Ady	ATP citrate lyase	[blue]	[blue]	1.5	1.8	2.0	1.1	147	100699_at	M77497	Cyp2i2	cytochrome P450, family 26, subfamily f,	[red]	[red]	1.8	1.1	-1.3	-1.3	750
99666_at	AW125431	Cs	citrate synthase	[red]	[blue]	1.3	-1.5	-1.1	-1.5	249	92814_at	AI114881	Cyp2j5	cytochrome P450, family 26, subfamily j,	[red]	[red]	2.0	3.3	1.7	-1.2	293
100289																					

Supplementary Figure

affy ID#	genbank ID#	symbol	gene name	fold changes				estimate				fold changes				estimate			
				R	W	T	+	R	W	T	+	db/db	affy ID#	genbank ID#	symbol	gene name	R	W	T
101307_at	Y10221	Cyp412	cytochrome P450, family 4, subfamily a,	-1.0	1.3	-1.2	-2.1	2749	160544_at	AJJ23066	Fabp5	fatty acid binding protein 5, epidermal	1.6	1.5	2.0	1.4	72		
101103_at	Y11638	Cyp414	cytochrome P450, family 4, subfamily a,	1.5	2.9	1.3	-9.2	1418	99867_at	U04827	Fabp7	fatty acid binding protein 7, brain	1.3	-1.0	2.2	1.0	22		
104129_at	AI462001	Cyp4113	cytochrome P450, family 4, subfamily f,	-1.5	1.6	-1.9	-1.1	182	93486_at	U15976	Slc27a1	solute carrier family 27 (fatty acid transporter),	1.2	2.9	1.1	-1.0	94		
100696_at	AF015811	AU04170	expressed sequence AU041707	1.5	1.2	-1.0	-3.3	32	100967_at	AF07275	Slc27a2	solute carrier family 27 (fatty acid transporter),	1.4	1.6	-1.1	-1.0	659		
104325_at	AI461631	Falp	Fat cell-specific low molecular weight protein	2.1	2.1	1.7	-1.6	198	97957_at	AF072759	Slc27a4	solute carrier family 27 (fatty acid transporter),	1.7	2.0	1.4	-1.1	46		
99592_f_at	AB030505	Rdh11	retinol dehydrogenase 11	1.5	1.5	1.0	-0.1	402	96231_at	AW123780	Bph1	Valacyclovir hydrolase precursor (Biphenyl	-1.3	-1.0	-2.3	-1.4	366		
103465_f_at	U60438	Saa2	serum amyloid A 2	1.1	-1.5	7.3	3.6	177	98984_f_at	D50430	Gpd2	glycerol phosphate dehydrogenase 2,	1.6	2.4	1.9	-1.2	169		
100333_at	M13521	Saa2	serum amyloid A 2	1.1	1.0	2.6	2.1	87	92798_at	U02980	Akp2	alkaline phosphatase 2, liver	1.0	1.6	1.4	-1.5	101		
102712_at	X03505	Saa3	serum amyloid A 3	-1.0	-1.1	1.7	2.0	32	102026_s_at	AB011000	Chkl	choline kinase-like	1.4	2.3	1.2	1.0	32		
92242_at	U65403	Saa4	serum amyloid A 4	-2.1	-2.6	-3.3	1.6	227	102027_s_at	AA204010	Chkl	choline kinase-like	1.5	2.2	1.3	-1.0	311		
104072_at	M23552	Apc8	apical membrane P-component	2.7	1.2	1.7	1.8	172	104371_at	AF078752	Gat1	diglycerol-CO ₂ -acyltransferase 1	1.6	1.5	1.1	-1.1	82		
103386_at	AW064123	Pte1	peroxisomal acyl-CoA thioesterase 1	1.3	1.8	1.2	-1.1	76	97525_at	U8403	Gyk	glycerol kinase	-1.1	1.9	1.1	1.2	243		
103646_at	X85983	Crat	carnitine acetyltransferase	3.0	6.6	2.8	-1.6	106	161753_f_at	AV290060	Gpd1	glycerol-3-phosphate dehydrogenase 1	1.7	1.1	-1.1	-1.1	40		
161989_f_at	AV28359	Crat	carnitine acetyltransferase	1.7	3.5	1.7	-1.2	38	92592_at	M25558	Gpd1	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	2.1	1.3	-1.1	-1.3	541		
95695_at	AB017172	Slc25a20	solute carrier family 25 (mitochondrial	1.6	2.7	1.2	-1.8	418	97511_at	AI846600	Mgll	monoglyceride lipase	1.5	3.5	1.2	-1.2	155		
100931_at	X73230	Arsa	arylsulfatase A	1.7	1.8	1.6	-1.3	110	101867_at	U11680	Gpan	glycerol-3-phosphate acyltransferase,	3.2	2.5	3.1	-1.3	701		
101891_at	Y09517	Hsd17b2	hydroxysteroid (17-beta) dehydrogenase 2	1.1	-1.3	1.6	1.9	142	104343_s_at	AI845798	Pta2g12a	phospholipase A ₂ , group XII	1.8	2.6	1.4	1.7	90		
97515_at	X89996	Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4	1.5	2.3	1.4	-1.6	936	98596_s_at	U15003	Stat9	silayl transferase 9	1.2	2.0	1.3	-1.4	404		
102123_z_at	Z31689	Lip1	lysosomal acid lipase 1	1.4	1.7	1.6	-1.3	95	161970_f_at	AV371169	Hmgcl	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	1.5	1.8	1.2	-1.4	123		
104273_at	U95215	Baat	bile acid-Coenzyme A: amino acid	-1.3	-1.7	-1.7	-1.1	375	94324_at	U49878	Hmgcl	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	1.7	2.2	1.2	-1.6	484		
95283_at	AI173996	Abcc2	ATP-binding cassette, sub-family C	1.1	1.6	1.9	-1.1	157	92590_at	U12791	Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A	-1.3	1.2	-2.4	-2.4	395		
103689_f_at	AA83514	Abcc3	ATP-binding cassette, sub-family C	1.9	1.3	1.2	-0.2	388	93096_at	D16195	Gm1	granulin	-1.0	1.5	1.7	-1.6	300		
93407_at	AB028737	Abcc3	ATP-binding cassette, sub-family C	-1.3	-1.5	-1.6	1.1	70	102839_at	D78354	Psrc1	phospholipid scramblase 1	1.4	1.6	2.0	1.2	41		
99404_at	L23754	Cyp7a1	cytochrome P450, family 7, subfamily a,	-3.0	-1.9	-1.3	-1.5	119	98589_at	M93275	Adrp	adipose differentiation related protein	2.3	4.1	2.8	-1.6	812		
161345_f_at	AV141027	Cyp7b1	cytochrome P450, family 7, subfamily b.	-2.1	-2.0	-2.6	12.8	65	102763_at	AF064748	S3-12	plasma membrane associated protein, S3-12	3.9	1.4	1.4	-1.2	30		
92898_f_at	U36993	Cyp7b1	cytochrome P450, family 7, subfamily b.	-2.6	-2.7	-3.6	12.8	108	104448_at	L47970	Mtp	microsomal triglyceride transfer protein	1.1	1.6	1.1	-1.2	56		
103284_at	AF090317	Cyp8b1	cytochrome P450, family 8, subfamily b,	-1.8	-2.9	-5.4	-1.5	1063	101173_at	Z50024	Pctp	phosphatidylcholine transfer protein	1.9	2.8	1.2	-2.6	79		
161668_f_at	AV140072	Por	cytochrome (cytochrome) oxidoreductase	-1.1	1.1	1.0	-1.5	145	100927_at	AV12890	Ptp	phospholipid transfer protein	2.2	7.6	4.6	-1.7	128		
9919_f_at	D17571	Por1	cytochrome (cytochrome) oxidoreductase	-1.1	1.4	1.2	-2.1	485	102696_s_at	AI747899	Ptpn1	phosphotyrosyl-protein transfer protein, beta	-1.6	-1.3	-1.1	-1.6	35		
100339_at	U95131	Slc10a1	solute carrier family 10 (sodium/bile acid	-1.7	-2.5	-4.0	-1.4	1965	92612_at	AJ223959	Slc27a5	solute carrier family 27 (fatty acid transporter),	-1.4	-2.1	-2.2	-1.3	1444		
100341_g_at	U95132	Slc10a1	solute carrier family 10 (sodium/bile acid	-1.8	-2.5	-4.2	-1.7	1414	100905_at	U37799	Scarb1	scavenger receptor class B, member 1	-1.4	-1.8	-1.5	1.3	302		
94325_at	AW124932	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A	1.3	3.0	-2.0	-1.1	230	96094_at	U79737	Apoa1	apolipoprotein A-I	1.4	1.6	1.9	1.9	5607		
102416_at	M64663	Cyp17a1	cytochrome P450, family 17, subfamily a,	-1.1	-1.4	-1.2	-1.7	376	100078_at	M64248	Apoa4	apolipoprotein A-IV	-2.0	-2.5	7.0	-3.8	583		
101659_at	M75886	Hsd3b2	hydroxysteroid dehydrogenase-2	-5.5	-6.6	-7.3	5.4	115	95727_at	AI785422	Apoa5	apolipoprotein A-V	-2.0	-2.1	-2.2	-1.4	2470		
98401_at	M77015	Hsd3b3	hydroxysteroid dehydrogenase-3	-1.3	-1.4	-1.6	1.0	84	95728_g_at	AI785422	Apoa5	apolipoprotein A-V	-2.0	-2.1	-1.9	-1.3	2444		
94795_at	L41519	Hsd3b5	hydroxysteroid dehydrogenase-5	-1.4	-1.3	-1.7	44.5	23	95729_at	AA674450	Apoa5	apolipoprotein A-V	-1.8	-2.2	-1.8	-1.4	964		
160104_at	AA824102	Hsd3b7	hydroxysteroid delta-5 steroid dehydrogenase, 3	-1.0	-1.0	-1.4	1.5	654	97887_at	Z22216	Apo2c	apolipoprotein C-II	1.5	1.3	1.9	-1.3	984		
102729_f_at	AF031170	Hsd3b6	hydroxysteroid dehydrogenase-6,	-1.2	-1.6	-1.5	1.3	102	96074_at	AI52149	ApoF	apolipoprotein F	-2.1	-2.2	-1.6	1.1	956		
95453_f_at	AF087687	S100a1	S100 calcium binding protein A1	1.6	-2.0	-1.9	-1.5	354	94318_at	Y11356	ApoH	apolipoprotein H	-1.3	-1.6	-1.8	-1.3	4081		
94284_at	AW122731	Dia1	diaphorase 1 (NADH)	1.5	1.1	1.4	1.0	640	93840_at	AA655303	ApoM	apolipoprotein M	-1.5	-1.7	-1.8	2.3	160		
160344_at	AB021289	Npc2	Niemann-Pick type C2	1.3	1.2	1.7	1.0	264	100332_s_at	AF093853	Prdx6	peroxiredoxin 6	-1.4	-1.5	-1.6	-1.6	555		
97132_at	AA189890	Soat2	sterol O-acetyltransferase 2	1.2	1.5	1.5	1.1	155	102820_at	M60358	Cyp2b13	cytochrome P450, family 2, subfamily b,	2.9	3.0	-1.2	-2.1	78		
94177_at	Y15733	Hsd17b7	hydroxysteroid (17-beta) dehydrogenase 7	-1.1	-1.7	-1.5	-1.3	47	103023_at	J05154	Loc4	leathin cholesterol acyltransferase	1.0	-1.3	-1.8	-1.4	741		
96895_at	U32684	Pon1	paraoxonase 1	-1.4	-2.1	-2.9	-1.5	1739	161703_f_at	AV003419	Anxa1	annexin A1	1.2	1.3	2.1	1.1	14		
98600_at	U141341	S100a11	S100 calcium binding protein A11 (calizzarin)	2.3	2.4	7.7	-2.2	29	93038_at	M69260	Scap	annexin A11	1.3	1.1	2.3	-1.0	21		
93042_at	D21207	Bzrp	benzodiazepine receptor, peripheral	1.7	1.4	1.5	1.1	591	100659_at	M14044	Anxa2	annexin A2	4.4	4.4	9.1	-1.4	112		
97248_at	X61431	Dbi	diapause binding inhibitor	1.7	2.2	1.6	-1.3	1771	101933_at	AA01633	Anxa3	annexin A3	1.0	1.1	1.7	1.0	16		
101923_at	U34277	Pla2g7	phospholipase A2, group VII	1.6	11.3	1.9	1.1	20	93083_at	D63423	Anxa5	annexin A5	1.9	1.4	2.9	-2.0	518		
102370_at	AA822174	Dhrs8	retinal short-chain dehydrogenase/reductase	1.9	6.5	1.9	-1.2	409	92539_at	M16465	S100a10	S100 calcium binding protein A10 (calactin)	1.3	1.1	2.0	-1.8	495		
96035_at	L47335	Bckdh	branched chain ketoacid dehydrogenase E1,	1.5	1.1	-1.0	1.2	311	160427_at	AW121668	Cds2	CDP-diacylglycerol synthase (phosphatidate	2.6	1.6	1.5	-1.0	15		
100803_at	U040380	Cyp128	cytochrome P450, family 2, subfamily d	-1.0	-2.1	-2.8	-1.0	31	106577_at	AF011336	Atp6	ATPase, class II, type 9A	1.4	1.6	1.3	-1.1	214		
102847_s_at	M19319	Cyp24a	cytochrome P450, family 2, subfamily a,	-1.5	-1.0	-1.6	-1.4	56	101944_at	U89352	Lyp1	lysophospholipase 1	-1.2	-1.1	-1.5	-1.7	645		
99463_at	X63023	Cyp3a13	cytochrome P450, family 3, subfamily a,	1.5	1.8	1.1	-1.6	336	96623_at	AI853172	Upgr	UDP-glucose ceramide glucosyltransferase	1.4	1.3	1.9	1.6	17		
100539_at	AI84129	Bach	cytobolic acyl-CoA thioester hydrolase	1.2	1.6	1.4	-1.1	58	92556_at	D45850	Arcf6	aldo-keto reductase family 1, member C6	-1.7	-2.3	-1.7	1.4	3513		
93754_at	AF030434	Ech1	enoyl-Coenzyme A hydratase 1, peroxisomal	1.4	2.7	1.2	-1.8	1147	94276_at	AF064635	Hsd17b12	hydroxysteroid (17-beta) dehydrogenase 12	2.4	2.8	1.4	1.2	262		
94507_at	U15977	Fad2	faecal Coenzyme A ligase, long chain 2	1.6	3.0	-1.3	-1.2	378	92437_at	AW074475	Tmr7sf2	transmembrane 7 superfamily member 2	1.0	-1.0	-1.6	-1.3	311		
100417_at	AB033887	Fad4	fatty acid-Coenzyme A ligase, long chain 4	1.3	1.7	2.0	1.1	15	97867_at	X83202	Hsd1b1	hydroxysteroid 11-beta-dehydrogenase 1	-1.8	-5.9	-4.0	-1.6	1624		
103649_at	AI640687	Hao3	hydroxyacid oxidase (glycotopeptidase 3)	-1.0	5.3	-1.2	-1.5	12	93316_at	AB017026	Osbp1a	oxysterol binding protein-like 1A	-1.1	1.0	-1.4	1.5	95		
94465_at	AB480013	Peci	peroxisomal delta-2, delta-2-enoyl-Coenzyme A	2.4	2.2	1.5	-1.6	1663	160737_at	AW06027	Lso	lanosterol synthase	1.4	2.1	1.0	-1.1	95		
95281_at	U07159	Acadm	acetyl-Coenzyme A dehydrogenase, medium	2.2	3.0	1.4	-1.5	837	95632_at	AI22653	Mvk	mevalonate kinase	-1.3	1.0	-1.7	1.0	101		
103401_at	L11163	Acads	acyl-Co																

Supplementary Figure

affy ID#	genbank ID#	symbol	gene name	fold changes				estimate				fold changes				estimate						
				R	W	T	+	R	W	T	+	db/db	affy ID#	genbank ID#	symbol	gene name	R	W	T	+	db/db	
2	retinol metabolism												102863_at	A1550530	9130423L	RIKEN cDNA 9130423L19 gene	-1.4	-1.3	-1.5	1.1	45	
101431_at	AF033195	Rdh5	retinol dehydrogenase 5					-1.3	-1.6	-1.4	-1.1	155	94995_at	A1584331	RIKEN cDNA A3030017C1 gene		1.9	2.1	1.9	1.0	24	
102797_at	X95281	Dhrs3	retinal short-chain dehydrogenase/reductase					-1.0	-1.5	-1.7	-1.2	238	103744_at	A1852760	A030014	RIKEN cDNA A930014C21 gene	1.8	1.6	1.2	-1.1	27	
178 unknown													99451_at	A1W06510	C230093	RIKEN cDNA C230093N12 gene		1.2	1.7	1.2	-1.4	129
17 unknown													95910_at	A1C584780	C5300081	RIKEN cDNA C3300081V15 gene		1.2	1.7	1.2	-1.3	78
92718_at	AI158810							1.1	1.1	1.6	1.0	42	97710_f_at	A1425990	C530046	RIKEN cDNA C530046L02 gene		1.5	-1.0	1.2	1.2	23
104761_at	AA612460	Antrb2	anthrax toxin receptor 2					-1.5	-1.6	-1.5	-1.4	62	103257_at	A1A690483	C730036	RIKEN cDNA C730036B01 gene		1.2	1.9	-1.0	-1.2	283
95749_at	AW122364	Armet	arginine-rich, mutated in early stage tumors					-1.2	-2.3	-1.3	1.4	347	103702_f_at	A1847632	C730048	RIKEN cDNA C730048C13 gene		-2.3	-6.6	-4.4	2.1	251
93984_at	AF002718	Atpi	ATPase inhibitor					1.0	1.1	1.4	-1.2	136	99366_at	A155336	E030024	RIKEN cDNA E030024M05 gene		-1.1	1.4	1.8	-1.0	11
162349_at	AV173028	C1qtnf1	C1q and tumor necrosis factor related protein					-1.2	-1.4	-1.6	-1.1	20	93627_at	A1B52287	E430019	RIKEN cDNA E430019N12 gene		-1.2	-1.2	-1.5	-1.1	112
160769_at	AW120606	Flana-gen	carcinoma related gene					-1.1	-1.6	-1.2	1.0	131	104621_at	A1W04606	F830029	RIKEN cDNA F830029L24 gene		-1.1	-1.1	-1.6	1.1	426
103817_at	AJ006469	Crtap	cartilage associated protein					1.1	1.5	1.6	-1.1	66	103675_at	A1Y17793	Robo1	roundabout homolog 1 (Drosophila)		1.1	1.1	1.1	-1.7	22
93908_f_at	X16670	Ccmrl	CCR4 carbon catabolite response 4-like (S. <i>lactucae</i>)					1.0	-1.1	-1.4	-1.5	53	96237_at	A118905	Smaf1	small adipocyte factor 1		1.9	1.0	1.1	1.1	15
103391_at	AI988033	C430041	cDNA sequence BC037135					1.1	1.1	-1.8	-1.2	124	97817_at	AW121136	Spec1	small protein effector 1 of Cdc42		1.2	1.2	1.6	-1.1	68
97928_at	AW045278	Cln6	ceroid-lipofuscinosis, neuronal 6					1.2	1.2	1.7	1.0	47	99127_at	XG1506	Sc10	spinocerebellar atrophy 10 homolog (human)		1.2	2.0	1.2	-1.0	185
94061_at	M13018	Crip1	cysteine-rich protein 1 (intestinal)					1.1	1.2	2.5	-1.0	32	94515_at	A1W08268	Sqrld	sulfide quinone reductase-like (yeast)		-1.0	-1.5	-1.5	-1.3	153
96134_at	A755260	Dp111	deleted in polyposis 1-like 1					1.2	1.4	-1.3	-1.8	2246	95104_at	U00674	Sdc2	syndecan 2, PRECURSOR (FIBROGLYCAN)		-1.5	-1.9	-1.8	1.1	171
96862_at	AI842936	Ga17	dendritic cell protein Ga17					-1.1	1.5	-1.1	1.0	130	102315_at	A1W24570	Tex292	tess expressed gene 292		-1.6	-1.1	-1.2	1.2	45
94967_at	A1851365	D19Wsu1	DNA segment, Chr 19, Wayne State University					2.4	1.9	1.6	-1.3	47	96773_at	AW125408	Txnd4	thioredoxin domain containing 4 (endoplasmic reticulum)		-1.3	-1.6	-1.5	1.1	113
104116_at	AW124049	D5E0759	DNA segment, Chr 5, ERATO Doi 593,					1.1	1.7	1.4	1.2	137	104755_at	A1J24278	Tripl	TNFIP3 interacting protein 1		1.3	1.3	1.5	-1.0	73
97770_s_at	AA733372	D6Wsu17	DNA segment, Chr 6, Wayne State University					-1.0	1.1	1.2	2.0	30	93538_at	A1W28036	Trap	Traf and Trt receptor associated protein		1.8	1.4	-1.3	-1.3	37
95709_at	AW012491	D7Wsu86	DNA segment, Chr 7, Wayne State University					-1.2	-1.5	-1.4	-1.0	910	160162_at	A1852545	Tagln2	transgelin 2		1.0	1.1	2.1	-1.0	131
98503_at	U53586	Evi5	ectopic viral integration site 5					-1.3	-2.2	-1.6	-1.0	116	95431_at	A1A623426	Tomm70a	translocase of outer mitochondrial membrane		-1.0	1.6	1.1	1.3	38
103531_f_at	A1049144		endoplasmic oxidoreductase 1 beta					-1.7	-1.9	-1.6	-1.3	98	95642_at	A1B35858	Tpm4	topomyosin		1.0	1.3	2.1	1.1	53
95366_at	AI847050	AA408111	EST AA408112					1.4	1.7	1.2	-1.1	81	104177_at	A1A204579	Vtg1-pnt	viral hemorrhagic septicemia virus (VHSV) gene		1.1	-1.0	1.5	1.7	29
98948_at	A1785289	Ns-pendin	EST C77032					-1.0	1.2	1.5	-1.1	41	100522_s_at	A1U2454	Wbp5	WW domain binding protein 5		-1.4	-1.0	2.2	2.1	50
97918_at	A1A62387	A5A3674	expressed sequence AA536743					1.4	1.4	2.2	-1.3	20	104583_at	A1J27492	Zdhnc8	zinc finger, DHH domain containing 6		-1.3	-1.3	-1.6	1.2	56
94359_at	AI849556	AA96055	expressed sequence AA960558					1.2	1.3	1.7	-1.1	65	104588_at	A1Z55961	Hamp	RIKEN cDNA 1810073K19 gene		-1.8	-2.8	-4.0	-1.1	1183
103400_at	D50523	D50523	expressed sequence AI316822					-1.0	1.1	1.0	1.7	27	96122_at	A1W049733	C210016A	RIKEN cDNA C210016A09 gene		1.2	1.5	-1.4	-1.6	1002
98849_at	C85252	A553587	expressed sequence AI553587					1.5	1.8	1.9	-1.0	1491	100516_at	A1B30821	Chk	choline kinase		1.1	1.1	1.5	1.1	64
93963_at	A200748	A1661017	expressed sequence AI661017					1.4	1.5	1.6	-1.0	17	160509_at	AF057156	Spr1a	small proline-rich protein 1A		1.1	1.8	10.6	1.0	54
103227_at	AI788959	A1788959	expressed sequence AI788959					1.2	1.6	1.4	1.3	402	95701_at	A1W24059	Wbp1	WW domain binding protein 5		-1.6	-1.7	-2.0	1.0	75
104413_at	AI834974	A1834974	expressed sequence AI834974					1.0	1.5	1.2	-1.1	311	103714_at	A1265638	0610009A	RIKEN cDNA 0610009A07 gene		-1.6	-1.8	-1.7	1.3	149
103429_f_at	AIW125330	A1O24210	expressed sequence AL024210					-1.2	-1.3	-1.5	-1.1	157	161122_f_at	A1V032952	NADH dehydrogenase (ubiquinone) 1		1.1	2.1	-1.1	-1.0	217	
104333_at	U69488	G7e-pend	G7e protein					1.1	2.1	3.8	1.0	18	160648_at	A1J047076	Flag1	flagellin-like 1		1.0	2.1	1.9	1.1	23
101444_at	US1716	GI(ROSA)	gene trap ROSA 26 antisense, Philippe					-1.1	1.5	1.2	1.0	68	101537_at	A1F04435	E51	esterase 1		-2.0	-3.8	-2.0	1.3	670
93028_at	X58196	H19	H19 fetal liver mRNA H19, imprinted					1.1	1.2	1.6	1.4	21	93534_at	X53929	Dcn	decorin		-1.5	-1.5	1.0	-1.2	567
93276_at	U901213	H1n	hematological and neurological expressed					1.3	1.1	1.6	1.1	48	103704_at	A1W047625	2010305K	RIKEN cDNA 2010305K11 gene		1.3	1.9	1.1	-1.3	53
96117_f_at	AI843732	H13	histocompatibility 13					-1.0	-1.6	-1.1	1.2	200	100586_i_at	A1B34395	5730403B	RIKEN cDNA 5730403B10 gene		-1.3	-1.4	-1.6	1.1	47
93824_at	AI182073	J734234	hypothetical protein J734234M3					-1.4	-2.1	-1.4	1.3	3295	95621_at	A1A60367	9030623	RIKEN cDNA 9030623C06 gene		1.1	1.8	1.5	1.0	24
161214_f_at	AV162528	MGC4743	hypothetical protein MGC4743					-1.1	-1.2	-1.1	1.7	17	103407_at	A1B85838	1300017	RIKEN cDNA 1300017Q12 gene		-1.2	-1.6	-2.1	1.3	878
94428_at	AI842686	Ilvb	IlvB (bacterial acetolactate synthase)-like					1.1	1.6	1.2	-1.4	60	98915_at	A1B49082	RNF149	ring finger protein 149		-1.6	-1.5	1.0	-1.4	133
95622_at	AW123907	Klhd2	kelch domain containing 2					1.1	1.7	1.6	2.0	20	102035_at	A1F037044	Tpm1	thiopurine S-methyltransferase		1.1	-1.2	-1.5	-1.3	444
96938_at	AB208071	Keg1	kidney expressed gene 1					-2.2	-2.1	-2.0	4.0	158	94536_s_at	A1B34317	2900073G15 gene		1.1	1.0	1.7	-1.5	691	
98123_at	AW226981	Kat2	kyurenine aminotransferase II					-1.2	-1.0	-1.9	1.3	63	95417_at	A1I17848	Mgat2	mannosidase acetylglucosaminyltransferase 2		-1.5	-1.7	-1.6	1.2	39
98440_at	AA567101	Lbd4h	leukotriene B4 12-hydroxydehydrogenase					-1.2	-1.1	-1.1	-1.5	28	97351_at	A1W23567	cytochrome c oxidase subunit Ib		1.0	3.7	1.3	-1.2	13	
96012_f_at	AI853567	Matr3	matrin 3					-1.2	-1.1	-1.1	1.7	101	97352_f_at	A1W23567	cytochrome c oxidase subunit Ib		1.2	8.2	1.6	1.0	28	
160119_at	AI845538	MCC3	matrix gamma-carboxylglutamate (gla) protein					1.1	-1.2	-1.0	1.6	58	103442_at	A1B43399	LOC2168	cDNA sequence BC03479		1.9	1.7	1.2	-1.0	123
103100_at	AI785434	Mus	musculus	clone IMAGE:5372338				1.5	1.6	1.2	-1.1	27	93909_f_at	X04120	Wdfc	WAP four-disulfide bond core domain protein 2		1.9	1.2	1.4	-1.2	39
100880_at	AA816121	Mdn1r	Mus musculus diabetic nephropathy-related					1.1	-1.1	1.0	1.6	22	161504_i_at	A1V27302	D10Ert2d	DNA segment, Chr 10, ERATO Doi 214,		1.1	1.1	1.1	1.5	16
103203_f_at	AW224743	Mig6	mitogen-inducible gene 6 protein homolog					1.1	-1.1	1.5	1.2	11	104417_at	A1T3533	AI876593	expressed sequence AI876593		1.1	1.1	1.1	1.5	16
103204_f_at	AW224749	Mig6	mitogen-inducible gene 6 protein homolog					1.1	-1.1	1.5	1.2	11	104617_at	A1O24964	06100059	RIKEN cDNA 06100059N1 gene		-1.1	-1.2	-1.3	-1.6	795
96556_g_at	C81463	Mus	Mus musculus transcribed sequence with					-1.4	-2.1	-1.6	-1.2	28	95026_at	A1W047688	0610039	RIKEN cDNA 0610039N19 gene		1.8	6.3	1.5	-3.5	414
100944_at	AA958903	Mus	Mus musculus transcribed sequences					-1.0	-1.9	-1.4	1.1	169	97514_at	A1W046438	1810063B05	RIKEN cDNA 1810063B05 gene		1.0	1.5	1.0	-1.8	131