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(54) Title: PROTEINS INVOLVED IN THE REGULATION OF ENERGY HOMEOSTASIS

(57) Abstract: The present invention discloses novel uses for energy homeostasis regulating proteins and polynucleotides encoding these in the diagnosis, study, prevention, and treatment of metabolic diseases and disorders.

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Proteins involved in the regulation of energy homeostasis**Description**

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This invention relates to the use of nucleic acid sequences encoding foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous proteins, to the use of polynucleotides encoding these, and to the use of modulators/effectors of the proteins and polynucleotides in the diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation, for example, but not limited to, metabolic diseases or dysfunctions such as obesity, metabolic syndrome, diabetes mellitus, eating disorder, cachexia, pancreatitis, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.

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There are several metabolic diseases of human and animal metabolism, eg., obesity and severe weight loss, that relate to energy imbalance where caloric intake versus energy expenditure is imbalanced. Obesity is one of the most prevalent metabolic disorders in the world. It is still a poorly understood human disease that becomes more and more relevant for western society. Obesity is defined as a body weight more than 20% in excess of the ideal body weight, frequently resulting in a significant impairment of health. Obesity may be measured by body mass index, an indicator of adiposity or fatness. Further parameters for defining obesity are waist circumferences, skinfold thickness and bioimpedance. Obesity is associated with an increased risk for cardiovascular disease, hypertension, diabetes, hyperlipidaemia and an increased mortality rate. Besides severe risks of illness, individuals suffering from obesity are often isolated socially.

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Obesity is influenced by genetic, metabolic, biochemical, psychological, and behavioral factors, and can be caused by different reasons such as non-insulin dependent diabetes, increase in triglycerides, increase in carbohydrate bound energy and low energy expenditure. As such, it is a complex disorder that must be addressed on several fronts to achieve lasting positive clinical outcome. Since obesity is not to be considered as a single disorder but as a heterogeneous group of conditions with (potential) multiple causes, it is also characterized by elevated fasting plasma insulin and an exaggerated insulin response to oral glucose intake (Kolterman O.G., (1980) Clin. Invest 65: 1272-1284). A clear involvement of obesity in type 2 diabetes mellitus can be confirmed (Kopelman P.G., (2000) Nature 404: 635-643).

Hyperlipidemia and elevation of free fatty acids correlate clearly with the metabolic syndrome, which is defined as the linkage between several diseases, including obesity and insulin resistance. This often occurs in the same patients and are major risk factors for development of type 2 diabetes and cardiovascular disease. It was suggested that the control of lipid levels and glucose levels is required to treat type 2 diabetes, heart disease, and other occurrences of metabolic syndrome (see, for example, Santomauro A.T. et al., (1999) Diabetes, 48: 1836-1841 and Lakka H.M., (2002) JAMA 288: 2709-2716).

The molecular factors regulating food intake and body weight balance are incompletely understood. Even if several candidate genes have been described which are supposed to influence the homeostatic system(s) that regulate body mass/weight, like leptin or the peroxisome proliferator-activated receptor-gamma co-activator, the distinct molecular mechanisms and/or molecules influencing obesity or body weight/body mass regulations are not known. In addition, several single-gene mutations resulting in obesity have been described in mice, implicating genetic factors in the etiology of obesity (Friedman J.M. and Leibel R.L., (1992) Cell 69:

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217-220). In the obese (ob) mouse, a single gene mutation (obese) results in profound obesity, which is accompanied by diabetes (Friedman J.M. et. al., (1991) Genomics 11: 1054-1062).

5 Therefore, the technical problem underlying the present invention was to provide for means and methods for modulating/effecting (pathological) metabolic conditions influencing body-weight regulation and/or energy homeostatic circuits. The solution to said technical problem is achieved by providing the embodiments characterized in the claims. Accordingly, the
10 present invention relates to novel functions of proteins and nucleic acids encoding these in body-weight regulation, energy homeostasis, metabolism, and obesity. The proteins disclosed herein and polynucleotides encoding these are thus suitable to investigate metabolic diseases and disorders. Further new compositions are provided that are useful in
15 diagnosis, treatment, and prognosis of metabolic diseases and disorders as described.

So far, it has not been described that the proteins of the invention and homologous proteins are involved in the regulation of energy homeostasis and body-weight regulation and related disorders, and thus, no functions in
20 metabolic diseases and dysfunctions and other diseases as listed above have been discussed. In this invention, we demonstrate that the correct gene dose of a protein of the invention is essential for maintenance of energy homeostasis. A genetic screen was used to identify that mutations
25 of a gene encoding a protein of the invention or a homologous gene causes changes in the metabolism, in particular related to obesity, which is reflected by a significant change in the triglyceride content, the major energy storage substance.

30 Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described as

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these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention that will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies that are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure.

The present invention discloses that foraging (GadFly Accession Number CG10033), CG5535 (GadFly Accession Number), CG14217 (GadFly Accession Number), Klp67A (GadFly Accession Number CG10923), CG10133 (GadFly Accession Number), CG3967 (GadFly Accession Number), CG32048 (GadFly Accession Number), CG13625 (GadFly Accession Number), Baldspot (GadFly Accession Number CG3971), CG31605 (GadFly Accession Number), fusilli (GadFly Accession Number CG8205), or schnurri (GadFly Accession Number CG7734) homologous proteins (herein referred to as "proteins of the invention" or "a protein of the invention") are regulating the energy homeostasis and fat metabolism, especially the metabolism and storage of triglycerides, and polynucleotides, which identify and encode the proteins disclosed in this invention. The invention also relates to vectors, host cells, and recombinant methods for producing the polypeptides and polynucleotides of the invention. The invention also relates to the use of these compounds and effectors/modulators thereof, e.g. antibodies, biologically active nucleic

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- acids, such as antisense molecules, RNAi molecules or ribozymes, aptamers, peptides or low-molecular weight organic compounds recognizing said polynucleotides or polypeptides, in the diagnosis, study, prevention, and treatment of metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.
- Cyclic GMP-dependent protein kinase (PKG) 1 and 2 play a role in several physiological processes including intestinal fluid secretion, ossification, cell adhesion and smooth muscle function (see, Pfeifer A. et al., (1996) Science 274(5295):2082-2086; Talts J. F. et al., (1998) Ann N Y Acad Sci. 857:74-85). PKG1 is a specific mediator of the NO/cGMP effects in murine smooth muscle. Loss of PKG1 abolishes nitric oxide (NO)/cGMP-dependent relaxation of smooth muscle, resulting in severe vascular and intestinal dysfunctions (see, for example, Pfeifer A. et al., (1998) EMBO J 17(11):3045-3051; Ny L. et al., (2000) Br J Pharmacol 129:395-401). Intracellular production of nitric oxide (NO) is thought to mediate the pancreatic B-cell-directed cytotoxicity of cytokines in insulin-dependent diabetes mellitus, and recent evidence has indicated that this may involve induction of apoptosis. A primary effect of NO is to activate soluble guanylyl cyclase leading to increased cGMP levels and this effect has been demonstrated in pancreatic B-cells, although no intracellular function has been defined for islet cGMP. An inhibitor of PKG, KT5823, suppressed IL-1 β -induced apoptosis, suggesting the involvement of a PKG-dependent pathway in the apoptotic process (Zaitsev S. V. et al., (2001) Diabetes 50 Suppl 1:S70-76).
- Transporters for cationic amino acids in animal cells differ in specificity, cation dependence, and physiological role. Two families of proteins have been identified: 1) cationic amino acid transporters (CAT) and 2)

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broad-scope transport proteins (BAT). In the CAT family, three genes encode for four different isoforms (CAT-1, CAT-2A, CAT-2(B) and CAT-3); these are approximately 70-kDa proteins with multiple transmembrane segments (12-14), and despite their structural similarity, they differ in tissue distribution, kinetics, and regulatory properties (Deves R. and Boyd C.A., (1998) *Physiol Rev* 78(2):487-545).

The mCAT-1 gene encodes a basic amino acid transporter that also acts as the receptor for murine ecotropic leukemia viruses. Targeted mutagenesis in embryonic stem cells has been used to introduce a germ-line null mutation into this gene. This mutation removes a domain critical for virus binding and inactivates amino acid transport activity. Homozygous mutant pups generated from these cells were approximately 25% smaller than normal littermates, very anemic, and died on the day of birth. Peripheral blood from homozygotes contained 50% fewer red blood cells, reduced hemoglobin levels, and showed a pronounced normoblastosis. Histological analyses of bone marrow, spleen, and liver showed a decrease in both erythroid progenitors and mature red blood cells. Thus, mCAT-1 not only appears to be the sole receptor for a group of murine ecotropic retroviruses associated with hematological disease but also plays a critical role in both hematopoiesis and growth control during mouse development (Perkins C. P. et al., (1997) *Genes Dev* 11(7):914-925). In day 17 placentae of mice that carried a null gene mutation for insulin-like growth factor II (IGF-II), the in vivo expression of Na⁺-independent cationic amino acid transporter protein 1 (CAT1) mRNA was increased. This indicates that IGF-II downregulates the expression of CAT1 (Matthews J. C. et al., (1999) *Placenta* 1999 Nov;20(8):639-650).

The thousand and one-amino acid protein kinase 1 (TAO1) is highly expressed in brain. TAO1 selectively activates mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinases (MEKs) 3, 4, and 6 of the stress-responsive mitogen-activated protein kinase pathways in

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vitro. The activation of and binding to MEK3 by TAO1 implicates TAO1 in the regulation of the p38-containing stress-responsive MAP kinase pathway. TAO proteins lie in stress-sensitive kinase cascades and define a mechanism by which these kinases may organize downstream targets. (see, Hutchison M. et al., (1998) J Biol Chem 273(44):28625-28632; 5 Chen Z. et al., (1999) J Biol Chem 274(40):28803-28807). Prostate-derived STE20-like kinase (PSK), a member of the sterile 20 (STE20) family of serine-threonine kinases activates the c-Jun N-terminal kinase mitogen-activated protein kinase pathway and regulates the actin 10 cytoskeleton organization (Moore T.M. et al., (2000) J Biol Chem 275(6):4311-4322).

Intracellular transport mediated by kinesin superfamily proteins (KIFs) is a highly regulated process. The members of the kinesin superfamily are 15 involved in microtubule-based movement of very different proteins, e.g. mannose-6-phosphate receptor, or even organelles, e.g. mitochondria, and vesicles (see, for example, Nakagawa T. et al., (2000) Cell 103(4):569-581; Tanaka Y. et al., (1998) Cell 93(7):1147-1158; Yonekawa Y. et al., (1998) J Cell Biol 1998 Apr 20;141(2):431-41). Some 20 members are also essential for axonal transport and endocytosis, e.g. clathrin-mediated (see, for example, Nakamura Y. et al., (1994) Neurosci Lett 180(1):25-28).

Phospholipase A2 (PLA2) enzymes are found in all living species and form 25 a diverse family of enzymes. PLA2 enzymes are critical regulators of prostaglandin and leukotriene synthesis and can directly modify the composition of cellular membranes. PLA2 enzymes release fatty acids and lysophospholipids, including the precursor of platelet-activating factor (PAF) from phospholipids. Free fatty acids, eicosanoids, lysophospholipids and PAF are potent regulators of inflammation, reproduction and 30 neurotoxicity (Bonventre J. V. et al., (1997) Nature 390(6660):622-625). PLA2 are involved in endocytosis in different organs and cell types (e.g.

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macrophages, neurons) and in the production of phospholipid-derived second messengers of phagocytosis (e.g. prostaglandins) (Lennartz M. R., (1999) *Int J Biochem Cell Biol.* 31(3-4):415-430).

5 Cytosolic PLA2 are found in small amounts within the cell and play a key role in the biosynthetic pathway leading to the formation of the platelet activating factors and the eicosanoids (Sharp J. D. et al., (1991) *J Biol Chem* 266(23):14850-14853). Cytosolic PLA2 is important for macrophage production of inflammatory mediators, fertility, and in the
10 pathophysiology of neuronal death after transient focal cerebral ischaemia, and plays a non-redundant role in allergic responses and reproductive physiology (see, Bonventre J. V. et al., supra; Uozumi N. et al., (1997) *Nature* 390(6660):618-622). There are at least three different phospholipase A2, group IV genes in man and mouse, PLA2G4A,
15 PLA2G4B, and PLA2G4C (Six D. A. and Dennis E. A., (2000) *Biochim Biophys Acta.* 1488(1-2):1-19). Cytosolic PLA2 preferentially releases arachidonic acid from phospholipids and PLA2G4A is regulated by changes in intracellular calcium concentration and by phosphorylation by mitogen-activated protein kinases (MAP kinases) (Clark J. D. et al., (1995)
20 *J Lipid Mediat Cell Signal* 12(2-3):83-117). Deletion of the group IV cytosolic PLA2 suppresses adenomatous polyposis coli (multiple intestinal neoplasia) (Apc(Min))-induced tumorigenesis (Hong K.H. et al., (2001) *Proc Natl Acad Sci U S A* 98(7):3935-3939).

25 Acyl-CoA N-acyltransferases are modifying enzymes that transfer fatty acids on specific substrates like histones, aminoglycoside, proteins (e.g. serotonin), cholesterol, other sterols, glucosamine-phosphate, diacylglycerol, glycerol-3-phosphate (see, for example, Farese R. V., (1998) *Curr Opin Lipidol* 9(2):119-123; Chen H. C. and Farese R. V., (2000) *Trends Cardiovasc Med* 10(5):188-192; Dircks L. K. and Sul H. S., (1997) *Biochim Biophys Acta* 1348(1-2):17-26). Acyltransferases are involved in fatty acid
30 remodeling of phospholipids and metabolism of bioactive lipids in

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mammalian cells (Yamashita A. et al., (1997) J Biochem (Tokyo) 122(1):1-16). As shown in this invention, the Drosophila gene CG3967 (GadFly Accession Number), encodes for a protein that contains an acyl-CoA N-acyltransferase domain, which is most homologous to the splice variants of the human hypothetical protein FLJ13158 (GenBank
5 Accession Numbers XP_042375.2 and NP_079185.1 for the proteins, XM_042375 and NM_024909 for the cDNAs). No functional data are available for these proteins.

10 Nitric oxide (NO) produced by neuronal nitric oxide synthase (nNOS) is important for N-methyl-D-aspartate (NMDA) receptor-dependent neurotransmitter release, neurotoxicity, and cyclic GMP elevations. The coupling of NMDA receptor-mediated calcium influx and nNOS activation is postulated to be due to a physical coupling of the receptor and the enzyme
15 by an intermediary adaptor protein, PSD95, through a unique PDZ-PDZ domain interaction between PSD95 and nNOS. The nNOS adaptor protein CAPON interacts with the nNOS PDZ domain through its C terminus. CAPON competes with PSD95 for interaction with nNOS, and overexpression of CAPON results in a loss of PSD95/nNOS complexes in
20 transfected cells. CAPON may influence nNOS by regulating its ability to associate with PSD95/NMDA receptor complexes (Jaffrey S. R. et al., (1998) Neuron 20(1):115-124). CAPON might be involved in worm muscle degeneration (Gieseler K. et al., (2000) Curr Biol 10(18):1092-1097). The nNOS adaptor protein CAPON interacts selectively with Dexras1, a
25 brain-enriched member of the Ras family of small monomeric G proteins, and with synapsins I, II, and III (see, for example, Fang M. et al., (2000) Neuron 28(1):183-193; Jaffrey S. R. et al., (2002) Proc Natl Acad Sci U S A 99(5):3199-3204). Diabetic gastropathy in mice reflects an insulin-sensitive reversible loss of nNOS. nNOS expression and pyloric
30 function are restored to normal levels by insulin treatment. In diabetic animals, delayed gastric emptying can be reversed with a phosphodiesterase inhibitor, sildenafil. (Watkins C. C. et al., (2000) J Clin

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Invest 106 (3):373-384). The lack the neuronal nitric oxide synthase (NOS) gene activity leads to the development of grossly enlarged stomachs, with hypertrophy of the pyloric sphincter and the circular muscle layer. This phenotype resembles the human disorder infantile pyloric stenosis, in which gastric outlet obstruction is associated with the lack of NDP neurons in the pylorus (Huang P. L. et al., (1993) Cell 75(7):1273-1286).

Basigin is a component of the blood-brain barrier (see, for example, Schlosshauer B., (1993) Bioessays 15(5):341-346; Igakura T. et al., (1996) Biochem Biophys Res Commun 224:33-36). Basigin, also named extracellular matrix metalloproteinase inducer (EMMPRIN), stimulates the production of interstitial collagenase, gelatinase A, and stromelysin-1 by fibroblasts (DeCastro R. et al., (1996) J Invest Dermatol 106(6):1260-1265). Basigin is an important cell-surface molecule involved in early embryogenesis and reproduction (Igakura T. et al., (1998) Dev Biol 194(2):152-65). Spermatogenesis in knockout mice lacking the basigin gene (Bsg (-/-)) was disrupted, and arrested at the metaphase of the first meiotic division leading to apoptosis (Toyama Y. et al., (1999) Anat Histol Embryol 28(3):205-213). Basigin is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression (Kirk P. et al., (2000) EMBO J 19(15):3896-3904). Basigin, which is closely colocalized with MCT1 on acinar cell membranes, was absent from islet cell membranes (Zhao C. et al., (2001) Diabetes 50(2):361-366). Basigin is a cell-surface receptor for CyPA and CyPB that are essential components of cyclophilin-initiated signaling cascades that culminates in ERK activation and chemotaxis (Yurchenko V. et al., (2001) Biochem Biophys Res Commun 288(4):786-788). Disruption of the 5A11/Basigin gene in the mouse results in photoreceptor degeneration and a corresponding decrease in electroretinogram amplitudes in mature mice (Ochrietor J. D. et al., (2002) Vision Res 42(4):447-453).

The Stromal cell-derived factor-1 may be implicated in the aggressiveness of the autoimmune process leading to type 1 diabetes (Dubois-Laforge D. et al., (2001) Diabetes 50(5):1211-1213).

- 5 Fatty acids are synthesized de novo from acetyl-CoA and malonyl-CoA through a series of reactions mediated by acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). In rodents, the principal fatty acid produced by FAS is palmitic acid (16:0). The fatty acids that accumulate in livers of sterol regulatory element-binding protein (SREBP) transgenic mice are 18
10 carbons rather than 16 carbons in length, suggesting that the enzymes required for the elongation of palmitic to stearic acid may be induced. The long chain fatty acyl elongase (LCE) mRNA is highly expressed in liver and adipose tissue of SREBP transgenic mice. Cells that overexpress LCE show enhanced addition of 2-carbon units to C12-C16 fatty acids. LCE catalyzes
15 the rate-limiting condensing step in this reaction. LCE expression is a component of the mammalian elongation system that converts palmitic to stearic acid (Moon Y. A. et al., (2001) J Biol Chem 276(48):45358-45366).
- 20 Cig30 (Cold inducible glycoprotein of 30 kDa; a synonym for Elovl3, Elongation of very long chain fatty acids protein 3) is implicated in the thermogenic function of brown adipose tissue of mice. Cig30 is expressed in brown fat and liver. Animals exposed to cold stress showed a selective elevation of Cig30 expression in brown fat. Similar increases were brought
25 about in two other conditions of brown fat recruitment, namely during perinatal development and after cafeteria diet. The Cig30 protein is involved in a pathway connected with brown fat hyperplasia (Tvrdik P. et al., (1997) J Biol Chem 272(50):31738-31746). Cig30 reverted the phenotype of the Flap endonuclease/Elongation of fatty acids protein 2
30 (fen1/elo2) mutant that has reduced levels of fatty acids in the C(20)-C(24) range. The dramatic induction of Cig30 expression during brown fat

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recruitment coincided with elevated elongation activity (Tvrdik P. et al., (2000) J Cell Biol 149(3):707-718).

As shown in this invention, the Drosophila gene with GadFly Accession
5 Number CG13625 encodes for a protein which is most homologous to
human unnamed protein product (GenBank Accession Number
BAB71593.1 for the protein, AK057832 for the cDNA) and to human
hypothetical protein MGC13125 (GenBank Accession Number
NP_116114.1 for the protein, NM_032725 for the cDNA). No functional
10 data are available for these proteins.

Drosophila fusilli encodes a RNA binding protein involved in the EGF
receptor signalling pathway. Fusilli interacts genetically with cactus, a
putative transcription factor, which is a component of the the EGF receptor
15 signalling pathway, too (Wakabayashi-Ito N. et al., (2001) Dev Biol
229(1):44-54). As shown in this invention, the Drosophila gene fusilli
(GadFly Accession Number CG8205) encodes for a protein which is most
homologous to human protein similar to fusilli; Enhancer of cactus
(GenBank Accession Number XP_007770.7 for the protein, XM_007770
20 for the cDNA) and to human hypothetical protein FLJ21918 (GenBank
Accession Number NP_079215.1 for the protein, NM_024939 for the
cDNA). No functional data are available for these proteins.

25 The zinc finger transcription factor kappa recognition component KRC
(HIVEP-3) is a hitherto unrecognized participant in the signal transduction
pathway leading from the TNF receptor to gene activation and may play a
critical role in inflammatory and apoptotic responses (Oukka M. et al.,
(2002) Mol Cell 9(1):121-131). TNF-alpha is a powerful autocrine and
30 paracrine regulator of adipose tissue, too (Coppack S. W., (2001) Proc
Nutr Soc 60(3):349-356). The Smad-Shn-2 (Smad-Hivep-2) complex is
involved in the thymic selection of T cells (Takagi T. et al., (2001) Nat

Immunol 2(11):1048-1053). The transcription factor PRDII-BF1 (HIVEP-1) activates human immunodeficiency virus type 1 gene expression (Seeler J. S. et al., (1994) J Virol. 68(2):1002-1009). TGF-beta is a key factor in experimental models of diabetic kidney disease as well as in patients with diabetic nephropathy. TGF-beta may also be involved in mediating the vascular dysfunction of diabetic kidney disease via its effects on the key intracellular calcium channel, the inositol trisphosphate receptor (IP(3)R) (Sharma K. and McGowan T. A., (2000) Cytokine Growth Factor Rev 11(1-2):115-123). Leptin controls the expression of TGF-beta1 and TGF-beta type II receptor (Wolf G. et al., (2002) Am J Kidney Dis 39(1):1-11).

Foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous proteins and nucleic acid molecules coding therefore are obtainable from insect or vertebrate species, e.g. mammals or birds. Particularly preferred are homologous nucleic acids, particularly nucleic acids encoding a human protein as described in Table 1.

The invention particularly relates to a nucleic acid molecule encoding a polypeptide contributing to regulating the energy homeostasis and the metabolism of triglycerides, wherein said nucleic acid molecule comprises

- (a) the nucleotide sequence encoding foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri or homologous nucleic acids, particularly nucleic acids encoding a human protein as described in Table 1, and/or a sequence complementary thereto,
- (b) a nucleotide sequence which hybridizes at 50°C in a solution containing 1 x SSC and 0.1% SDS to a sequence of (a),
- (c) a sequence corresponding to the sequences of (a) or (b) within the degeneration of the genetic code,

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- (d) a sequence which encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to the amino acid sequences of foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri protein, preferably of the human homologous proteins, particularly a human protein as described in Table 1,
- (e) a sequence which differs from the nucleic acid molecule of (a) to (d) by mutation and wherein said mutation causes an alteration, deletion, duplication and/or premature stop in the encoded polypeptide or
- (f) a partial sequence of any of the nucleotide sequences of (a) to (e) having a length of 15-25 bases, preferably 25-35 bases, more preferably 35-50 bases and most preferably at least 50 bases.

The invention is based on the finding that foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri and/or homologous proteins and the polynucleotides encoding these, are involved in the regulation of triglyceride storage and therefore energy homeostasis. The invention describes the use of these compositions for the diagnosis, study, prevention, or treatment of metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, liver fibrosis, or gallstones.

Accordingly, the present invention relates to genes with novel functions in body-weight regulation, energy homeostasis, metabolism, and obesity, functional fragments of said genes, polypeptides encoded by said genes or functional fragments thereof, and modulators/effectors thereof, e.g. antibodies, biologically active nucleic acids, such as antisense molecules,

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RNAi molecules, or ribozymes, aptamers, peptides or low-molecular weight organic compounds recognizing said polynucleotides or polypeptides.

5 The ability to manipulate and screen the genomes of model organisms such as the fly *Drosophila melanogaster* provides a powerful tool to analyze biological and biochemical processes that have direct relevance to more complex vertebrate organisms due to significant evolutionary conservation of genes, cellular processes, and pathways (see, for example, Adams M.D. et al., (2000) *Science* 287: 2185-2195). Identification of novel gene
10 functions in model organisms can directly contribute to the elucidation of correlative pathways in mammals (humans) and of methods of modulating them. A correlation between a pathology model (such as changes in triglyceride levels as indication for metabolic syndrome including obesity) and the modified expression of a fly gene can identify the association of
15 the human ortholog with the particular human disease.

In one embodiment, a forward genetic screen is performed in fly displaying a mutant phenotype due to misexpression of a known gene (see, St Johnston D., (2002) *Nat Rev Genet* 3: 176-188; Rorth P., (1996) *Proc Natl Acad Sci U S A* 93: 12418-12422). In this invention, we have used a
20 genetic screen to identify mutations that cause changes in the body weight, which are reflected by a significant change of triglyceride levels.

Obese people mainly show a significant increase in the content of
25 triglycerides. Triglycerides are the most efficient storage for energy in cells. In order to isolate genes with a function in energy homeostasis, several thousand proprietary and publicly available EP-lines were tested for their triglyceride content after a prolonged feeding period (see Examples and Figures for more detail). Lines with significantly changed triglyceride
30 content were selected as positive candidates for further analysis. The increase or decrease of triglyceride content due to the loss or gain of a gene function suggests gene activities in energy homeostasis in a dose

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dependent manner that controls the amount of energy stored as triglycerides.

5 In this invention, the content of triglycerides of a pool of flies with the same genotype was analyzed after prolonged feeding using a triglyceride assay. Male flies homozygous, hemizygous, or heterozygous for the integration of vectors for *Drosophila* EP-lines were analyzed in an assay measuring the triglyceride contents of these flies, illustrated in more detail in the Examples section. The results of the triglyceride content analysis are
10 shown in Figures 1, 6, 10, 14, 17, 20, 22, 24, 27, 31, 33, and 35.

Genomic DNA sequences were isolated that are localized adjacent to the EP vector integration. Using those isolated genomic sequences public
15 databases like Berkeley *Drosophila* Genome Project (GadFly; see also FlyBase (1999) *Nucleic Acids Research* 27: 85-88) were screened thereby identifying the integration site of the vectors, and the corresponding genes, described in more detail in the Examples section. The molecular organization of the genes is shown in Figures 2, 7, 11, 15, 18, 21, 23, 25,
20 28, 32, 34, and 36.

20 An additional screen using *Drosophila* mutants with modifications of the eye phenotype identified a modification of UCP activity by CG32048 and *schnurri*, thereby leading to an altered mitochondrial activity. An additional genetic screen uses a *Drosophila* line with ectopic expression of *adp* in the
25 developing eye thereby leading to a visible change of the eye phenotype. Modifications of the eye phenotype by coexpression of *schnurri* or CG32048 identified an interaction of *schnurri* and CG32048 with *adp*, a protein regulating, causing or contributing to obesity. Additionally, another genetic screen uses a *Drosophila* line with ectopic expression of *adipose*
30 (*adp*) mainly in the fatbody thereby leading to lethality. Modifications of the lethality phenotype by coexpression of CG3967, CG32048, or *schnurri* identified an interaction of CG3967, CG32048, and *schnurri* with *adp*.

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These findings suggest the presence of similar activities of these described homolog proteins in humans that provides insight into diagnosis, treatment, and prognosis of metabolic disorders.

- 5 The *Drosophila* genes and proteins encoded thereby with functions in the regulation of triglyceride metabolism were further analysed in publicly available sequence databases (see Examples for more detail) and mammalian homologs were identified.
- 10 The function of the mammalian homologs in energy homeostasis was further validated in this invention by analyzing the expression of the transcripts in different tissues and by analyzing the role in adipocyte differentiation. Expression profiling studies (see Examples for more detail) confirm the particular relevance of the protein(s) of the invention as
- 15 regulators of energy metabolism in mammals. Further, we show that the proteins of the invention are regulated by fasting and by genetically induced obesity. In this invention, we used mouse models of insulin resistance and/or diabetes, such as mice carrying gene knockouts in the leptin pathway (for example, ob (leptin) or db (leptin receptor) mice) to
- 20 study the expression of the protein of the invention. Such mice develop typical symptoms of diabetes, show hepatic lipid accumulation and frequently have increased plasma lipid levels (see Bruning J.C. et al, (1998) Mol. Cell. 2: 559-569).
- 25 Microarrays are analytical tools routinely used in bioanalysis. A microarray has molecules distributed over, and stably associated with, the surface of a solid support. The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, antibodies, or other chemical compounds on a substrate. Microarrays of polypeptides, polynucleotides,
- 30 and/or antibodies have been developed and find use in a variety of applications, such as monitoring gene expression, drug discovery, gene sequencing, gene mapping, bacterial identification, and combinatorial

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chemistry. One area in particular in which microarrays find use is in gene expression analysis (see Example 7). Array technology can be used to explore the expression of a single polymorphic gene or the expression profile of a large number of related or unrelated genes. When the expression of a single gene is examined, arrays are employed to detect the expression of a specific gene or its variants. When an expression profile is examined, arrays provide a platform for identifying genes that are tissue specific, are affected by a substance being tested in a toxicology assay, are part of a signaling cascade, carry out housekeeping functions, or are specifically related to a particular genetic predisposition, condition, disease, or disorder.

Microarrays may be prepared, used, and analyzed using methods known in the art (see for example, Brennan T.M., (1995) U.S. Patent No. US5474796; Schena M. et al., (1996) Proc. Natl. Acad. Sci. USA 93: 10614-10619; Baldeschwieler et al., (1995) PCT application WO9525116; Shalon T.D. and Brown P.O., (1995) PCT application WO9535505; Heller R.A. et al., (1997) Proc. Natl. Acad. Sci. USA 94: 2150-2155; Heller M.J. and Tu E., (1997) U.S. Patent No. US5605662). Various types of microarrays are well known and thoroughly described in Schena M., ed. (1999); DNA Microarrays: A Practical Approach, Oxford University Press, London.

Oligonucleotides or longer fragments derived from any of the polynucleotides described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques, which monitor the relative expression levels of large numbers of genes simultaneously as described below. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the

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activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents, which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

As determined by microarray analysis, the human foraging homolog cGMP-dependent protein kinase, type II (PRKG2), the human CG31605 homologs stromal cell derived factor receptor 1 (SDFR1) and basigin (BSG), the human Baldspot homolog ELOVL family member 6, elongation of long chain fatty acids (ELOVL6), and the human schnurri homolog immunodeficiency virus type I enhancer binding protein 1 (HIVEP1) show differential expression in human primary adipocytes. Thus, PRKG2, SDFR1, BSG, ELOVL6, and HIVEP1 are strong candidates for the manufacture of a pharmaceutical composition and a medicament for the treatment of conditions related to human metabolism, such as obesity, diabetes, and/or metabolic syndrome.

The invention also encompasses polynucleotides that encode a protein of the invention or a homologous protein. Accordingly, any nucleic acid sequence, which encodes the amino acid sequences of a protein of the invention or a homologous protein, can be used to generate recombinant molecules that express a protein of the invention or a homologous protein. In a particular embodiment, the invention encompasses a nucleic acid encoding *Drosophila* foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri, or human foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologs, preferably a human homologous protein as described in Table 1; referred to as the proteins of the invention. It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude

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of nucleotide sequences encoding the proteins, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. The invention contemplates each and every possible variation of nucleotide sequence that can be made by selecting combinations based on possible codon choices.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed nucleotide sequences, and in particular, those of the polynucleotide encoding foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri, or a homologous protein, preferably a human homologous protein as described in Table 1, under various conditions of stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex or probe, as taught in Wahl G.M. et al., (1987) *Methods Enzymol.* 152: 399-407 and Kimmel A.R., (1987) *Methods Enzymol.* 152: 507-511), and may be used at a defined stringency. Preferably, hybridization under stringent conditions means that after washing for 1 h with 1 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C, particularly for 1 h in 0.2 x SSC and 0.1% SDS at 50°C, preferably at 55°C, more preferably at 62°C and most preferably at 68°C, a positive hybridization signal is observed. Altered nucleic acid sequences encoding the proteins which are encompassed by the invention include deletions, insertions or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent protein.

The encoded proteins may also contain deletions, insertions or substitutions of amino acid residues, which produce a silent change and result in functionally equivalent proteins. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of the protein is retained.

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Furthermore, the invention relates to peptide fragments of the proteins or derivatives thereof such as cyclic peptides, retro-inverso peptides or peptide mimetics having a length of at least 4, preferably at least 6 and up to 50 amino acids.

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Also included within the scope of the present invention are alleles of the genes encoding a protein of the invention or a homologous protein. As used herein, an 'allele' or 'allelic sequence' is an alternative form of the gene, which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structures or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes, which give rise to alleles, are generally ascribed to natural deletions, additions or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence.

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The nucleic acid sequences encoding a protein of the invention or a homologous protein may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed, 'restriction-site' PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar G. et al., (1993) PCR Methods Applic. 2: 318-322). Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region (Triglia T. et al., (1988) Nucleic Acids Res. 16: 8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom M. et al., (1991) PCR Methods Applic. 1: 111-119). Another method which may be used to retrieve unknown sequences is that of Parker J.D. et al., (1991) Nucleic Acids Res. 19: 3055-3060. Additionally, one may use PCR, nested

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primers, and PROMOTERFINDER libraries to walk in genomic DNA (Clontech, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

5 In order to express a biologically active protein, the nucleotide sequences encoding the proteins or functional equivalents, may be inserted into appropriate expression vectors, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods, which are well known to those skilled in the art, may
10 be used to construct expression vectors containing sequences encoding the proteins and the appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook J. et al. (1989) Molecular Cloning, A Laboratory
15 Manual, Cold Spring Harbor Press, Plainview, N.Y. and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

In a further embodiment of the invention, natural, modified or recombinant
20 nucleic acid sequences encoding a protein of the invention or a homologous protein may be ligated to a heterologous sequence to encode a fusion protein. Heterologous sequences are preferably located at the N-and/or C-terminus of the fusion protein.

25 A variety of expression vector/host systems may be utilized to contain and express sequences encoding the proteins or fusion proteins. These include, but are not limited to, micro-organisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems
30 infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower

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mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or PBR322 plasmids); or animal cell systems.

5 The presence of polynucleotide sequences encoding a protein of the invention or a homologous protein in a sample can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of said polynucleotides. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences specific for the gene to detect transformants containing DNA or
10 RNA encoding the corresponding protein. As used herein 'oligonucleotides' or 'oligomers' refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplifier.

15 A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting polynucleotide sequences encoding a protein of the invention or
20 a homologous protein include oligo-labeling, nick translation, end-labeling of labeled RNA probes, PCR amplification using a labeled nucleotide, or enzymatic synthesis. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, Mich.); Promega (Madison Wis.); and U.S. Biochemical Corp., (Cleveland, Ohio).

25 Suitable reporter molecules or labels, which may be used for nucleic acid and protein assays, include radionuclides, enzymes, fluorescent, chemiluminescent or chromogenic agents as well as substrates, co-factors, inhibitors, magnetic particles, and the like.

30 Host cells transformed with nucleotide sequences encoding a protein of the invention may be cultured under conditions suitable for the expression and

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recovery of said protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides, which encode the protein may be designed to contain signal sequences, which direct secretion of the protein through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding the protein to nucleotide sequence encoding a polypeptide domain, which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAG extension/affinity purification system (Immunex Corp., Seattle, Wash.) The inclusion of cleavable linker sequences such as those specific for Factor XA or Enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the desired protein may be used to facilitate purification.

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Diagnostics and Therapeutics

The data disclosed in this invention show that the nucleic acids and proteins of the invention and effector/modulator molecules thereof are useful in diagnostic and therapeutic applications implicated, for example but not limited to, metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis. Hence, diagnostic and therapeutic uses for the nucleic acids and proteins of the invention are, for example but not limited to, the following: (i) protein therapy, (ii) small molecule drug target, (iii) antibody target

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(therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) diagnostic and/or prognostic marker, (v) gene therapy (gene delivery/gene ablation), (vi) research tools, and (vii) tissue regeneration in vitro and in vivo (regeneration for all these tissues and cell types composing these tissues and cell types derived from these tissues).

The nucleic acids and proteins of the invention and modulators/effectors thereof are useful in diagnostic and therapeutic applications implicated in various applications as described below. For example, but not limited to, cDNAs encoding a protein of the invention and particularly their human homologues may be useful in gene therapy, and the proteins of the invention and particularly their human homologues may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from, for example, but not limited to, in metabolic disorders as described above.

The nucleic acids encoding a protein of the invention, a homologous protein, or a functional fragment thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acids or the proteins are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention may be used in therapeutic or diagnostic methods.

For example, in one aspect, antibodies, which are specific for a protein of the invention or a homologous protein, may be used directly as a modulator/effector, e.g. an antagonist or an agonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express the protein. The antibodies may be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric single chain, Fab

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fragments, and fragments produced by a Fab expression library. Neutralising antibodies, (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

5 For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with the protein or any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. It is preferred that the peptides,
10 fragments or oligopeptides used to induce antibodies to the protein have an amino acid sequence consisting of at least five amino acids, and more preferably at least 10 amino acids.

Monoclonal antibodies to the proteins may be prepared using any
15 technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Köhler G. and Milstein C. (1975) Nature 256: 495-497; Kozbor D. et al. (1985) J. Immunol. Methods 81: 31-42; Cote
20 R.J. et al., (1983) Proc. Natl. Acad. Sci. 80: 2026-2030; Cole S.P. et al., (1984) Mol Cell Biochem. 62: 109-120).

In addition, techniques developed for the production of 'chimeric antibodies', the splicing of mouse antibody genes to human antibody genes
25 to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison S.L. et al., (1984) Proc. Natl. Acad. Sci. 81: 6851-6855; Neuberger M.S. et al (1984) Nature 312: 604-608; Takeda S. et al., (1985) Nature 314: 452-454). Alternatively, techniques described for the production of single chain antibodies may be adapted, using
30 methods known in the art, to produce single chain antibodies specific for a protein of the invention or a homologous protein. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by

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chain shuffling from random combinatorial immunoglobulin libraries (Kang A.S. et al., (1991) Proc. Natl. Acad. Sci. 88: 11120-11123). Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi R. et al., (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; Winter G. and Milstein C., (1991) Nature 349: 293-299).

Antibody fragments which contain specific binding sites for the proteins may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by Pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse W.D. et al., (1989) Science 246: 1275-1281).

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding and immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between the protein and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering protein epitopes are preferred, but a competitive binding assay may also be employed (Maddox D.E. et al., (1983) J. Exp. Med. 158: 1211-1216).

In another embodiment of the invention, the polynucleotides of the invention or fragments thereof or nucleic acid modulator/effector molecules such as aptamers, antisense molecules, RNAi molecules or ribozymes may be used for therapeutic purposes. In one aspect, aptamers, i.e. nucleic acid

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molecules, which are capable of binding to a protein of the invention and modulating its activity, may be generated by a screening and selection procedure involving the use of combinatorial nucleic acid libraries.

5 In a further aspect, antisense molecules may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding a protein of the invention or a homologous protein. Thus, antisense molecules may be used to modulate/effect protein
10 activity or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligomers or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding the proteins. Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses or from
15 various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods, which are well known to those skilled in the art, can be used to construct recombinant vectors, which will express antisense molecules complementary to the polynucleotides of the genes encoding a protein of
20 the invention or a homologous protein. These techniques are described both in Sambrook et al. (supra) and in Ausubel et al. (supra). Genes encoding a protein of the invention or a homologous protein can be turned off by transforming a cell or tissue with expression vectors, which express high levels of polynucleotides that encode a protein of the invention, or a
25 homologous protein, or a functional fragment thereof. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more
30 with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

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As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, e.g. DNA, RNA or PNA, to the control regions of the genes encoding a protein of the invention or a homologous protein, i.e., the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it cause inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee J.E. et al. (1994) *Gene* 149: 109-114.; Huber B.E. and Carr B. I., (1994) *Molecular and Immunologic Approaches*, Futura Publishing Co., Mt. Kisco, N.Y.). The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples, which may be used, include engineered hammerhead motif ribozyme molecules that can be specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding a protein of the invention or a homologous protein. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

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Nucleic acid effector molecules, e.g. antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase
5 phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding a protein of the invention or a homologous protein. Such DNA sequences may be incorporated into a variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA
10 constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues. RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or modifications in the nucleobase, sugar
15 and/or phosphate moieties, e.g. the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine, and wybutosine, as well as acetyl-,
20 methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and
25 equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome injections may be achieved using methods, which are well known in the art. Any of the therapeutic methods
30 described above may be applied to any suitable subject including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

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An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of a nucleic acid sequence or protein of the invention or a homologous nucleic acid sequence or protein, antibodies to a protein of the invention or a homologous protein, mimetics, agonists, antagonists or inhibitors of a nucleic acid sequence or protein of the invention or a homologous nucleic acid sequence or protein. The compositions may be administered alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone or in combination with other agents, drugs or hormones. The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations, which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. The pharmaceutical composition may be provided as a salt and can be formed

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with many acids. After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of proteins, such labeling would include amount, frequency, and method of administration.

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Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compounds, the therapeutically effective dose can be estimated initially
10 either in cell culture assays, e.g., of preadipocyte cell lines or in animal models, usually mice, rabbits, dogs or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful
15 doses and routes for administration in humans. A therapeutically effective dose refers to that amount of active ingredient, for example a protein of the invention or a homologous protein or nucleic acid sequence or a functional fragment thereof or an antibody, which is sufficient for treating a specific condition. Therapeutic efficacy and toxicity may be determined
20 by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions,
25 which exhibit large therapeutic indices, are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range
30 depending upon the dosage from employed, sensitivity of the patient, and the route of administration. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires

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treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors, which may be taken into account, include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week or once every two weeks depending on half-life and clearance rate of the particular formulation. Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

In another embodiment, antibodies which specifically bind to a protein of the invention may be used for the diagnosis of conditions or diseases characterized by or associated with over- or underexpression of a protein of the invention or a homologous protein or in assays to monitor patients being treated with a protein of the invention or a homologous protein, or modulators/effectors thereof, e.g. agonists, antagonists, or inhibitors. Diagnostic assays include methods which utilize the antibody and a label to detect the protein in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used several of which are described above.

A variety of protocols including ELISA, RIA, and FACS for measuring proteins are known in the art and provide a basis for diagnosing altered or

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abnormal levels of gene expression. Normal or standard values for gene expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibodies to the protein under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, but preferably by photometric means. Quantities of protein expressed in control and disease, samples e.g. from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

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In another embodiment of the invention, the polynucleotides specific for a protein of the invention or a homologous protein may be used for diagnostic purposes. The polynucleotides, which may be used, include oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which gene expression may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess gene expression, and to monitor regulation of protein levels during therapeutic intervention.

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In one aspect, hybridization with probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding a protein of the invention or a homologous protein or closely related molecules, may be used to identify nucleic acid sequences which encode the respective protein. The hybridization probes of the subject invention may be DNA or RNA and derived from the nucleotide sequence of the polynucleotide encoding a protein of the invention or a homologous protein or from a genomic sequence including promoter, enhancer elements, and introns of the naturally occurring gene. Means for producing specific hybridization probes for DNAs encoding a protein of the invention or a homologous protein include the cloning of nucleic acid sequences specific for a protein of the invention or a homologous protein into vectors for the

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production of mRNA probes. Such vectors are known in the art, commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, radionuclides such as ^{32}P or ^{35}S or enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences specific for a protein of the invention or homologous nucleic acids may be used for the diagnosis of conditions or diseases, which are associated with the expression of the protein. Examples of such conditions or diseases include, but are not limited to, metabolic diseases and disorders, including obesity or diabetes. Polynucleotide sequences specific for a protein of the invention or a homologous protein may also be used to monitor the progress of patients receiving treatment for metabolic diseases and disorders, including obesity or diabetes. The polynucleotide sequences may be used qualitative or quantitative assays, e.g. in Southern or Northern analysis, dot blot or other membrane-based technologies; in PCR technologies; or in dip stick, pin, ELISA or chip assays utilizing fluids or tissues from patient biopsies to detect altered gene expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences specific for a protein of the invention or homologous nucleic acids may be useful in assays that detect activation or induction of various metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis. The nucleotide sequences may be labeled by standard methods, and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a

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suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. The presence of altered levels of nucleotide sequences encoding a protein of the invention or a homologous protein in the sample indicates the presence of the associated disease. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disease associated with expression of a protein of the invention or a homologous protein, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence or a fragment thereof, which is specific for the nucleic acids encoding a protein of the invention or homologous nucleic acids, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease. Once disease is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to evaluate whether the level of expression in the patient begins to approximate that, which is observed in the normal patient. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to metabolic diseases such as described above the presence of an unusual amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease or may provide a means for detecting the disease prior to the appearance of actual

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clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the metabolic diseases or dysfunctions. Additional diagnostic uses for oligonucleotides designed from the sequences encoding a protein of the invention or a homologous protein may involve the use of PCR. Such oligomers may be chemically synthesized, generated enzymatically or produced from a recombinant source. Oligomers will preferably consist of two nucleotide sequences, one with sense orientation (5'.fwdarw.3') and another with antisense (3'.rarw.5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of a protein of the invention or a homologous protein include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby P.C. et al., (1993) J. Immunol. Methods, 159: 235-244; Duplaa C. et al., (1993) Anal. Biochem. 212: 229-236). The speed of quantification of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantification.

In another embodiment of the invention, the nucleic acid sequences which are specific for a protein of the invention or homologous nucleic acids may also be used to generate hybridization probes, which are useful for mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. Such techniques include FISH, FACS or artificial chromosome constructions, such as yeast artificial

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chromosomes, bacterial artificial chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price C.M., (1993) Blood Rev. 7: 127-134, and Trask B.J., (1991) Trends Genet. 7: 149-154. FISH is described in Verma R.S. and Babu A., (1989) Human
5 Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, N.Y.. The results may be correlated with other physical chromosome mapping techniques and genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of the gene encoding a protein of the
10 invention on a physical chromosomal map and a specific disease or predisposition to a specific disease, may help to delimit the region of DNA associated with that genetic disease.

The nucleotide sequences of the subject invention may be used to detect
15 differences in gene sequences between normal, carrier or affected individuals. For example, an analysis of polymorphisms, e.g. single nucleotide polymorphisms may be carried out. Further, in situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for
20 extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms or parts thereof, by physical mapping. This provides
25 valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti R.A. et al., (1988) Nature 336: 577-580), any sequences mapping to that area may represent
30 associated or regulatory genes for further investigation. The nucleotide sequences of the subject invention may also be used to detect differences

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in the chromosomal location due to translocation, inversion, etc. among normal, carrier or affected individuals.

5 In another embodiment of the invention, the proteins of the invention, their catalytic or immunogenic fragments or oligopeptides thereof, an in vitro model, a genetically altered cell or animal, can be used for screening libraries of compounds, e.g. peptides or low molecular weight organic compounds, in any of a variety of drug screening techniques. One can identify modulators/effectors, e.g. receptors, enzymes, proteins, ligands, or
10 substrates that bind to, modulate or mimic the action of one or more of the proteins of the invention. The protein or fragment thereof employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between a protein of the invention and the agent tested, may be measured.
15 Agents could also, either directly or indirectly, influence the activity of a protein of the invention.

In vivo, the enzymatic kinase activity of the unmodified polypeptides of foraging or CG14217 homologous kinase towards a substrate can be
20 measured. Activation of the kinases may be induced in the natural context by extracellular or intracellular stimuli, such as signaling molecules or environmental influences. One may generate a system containing foraging or CG14217 homologous kinase, may it be an organism, a tissue, a culture of cells or cell-free environment, by exogenously applying this stimulus or
25 by mimicking this stimulus by a variety of the techniques, some of them described further below. A system containing activated foraging or CG14217 homologous kinase may be produced (i) for the purpose of diagnosis, study, prevention, and treatment of diseases and disorders related to body-weight regulation and thermogenesis, for example, but not
30 limited to, metabolic diseases, (ii) for the purpose of identifying or validating therapeutic candidate agents, pharmaceuticals or drugs that influence the genes of the invention or their encoded polypeptides, (iii) for

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the purpose of generating cell lysates containing activated polypeptides encoded by the genes of the invention, (iv) for the purpose of isolating from this source activated polypeptides encoded by the genes of the invention.

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In one embodiment of the invention, one may produce activated foraging or CG14217 homologous kinase independent of the natural stimuli for the above said purposes by, for example, but not limited to, (i) an agent that mimics the natural stimulus; (ii) an agent, that acts downstream of the natural stimulus, such as activators of the foraging or CG14217 homologous kinase, phorbol ester, anisomycin, constitutive active alleles of the foraging or CG14217 homologous kinase itself as they are described or may be developed; (iii) by introduction of single or multiple amino acid substitutions, deletions or insertions within the sequence of foraging or CG14217 homologous kinase to yield constitutive active forms; (iv) by the use of isolated fragments of foraging or CG14217 homologous kinase. In addition, one may generate enzymatically active foraging or CG14217 homologous kinase in an ectopic system, prokaryotic or eukaryotic, in vivo or in vitro, by co-transferring to this system the activating components.

20

Many assays for phospholipase activity are known in the art and may be used with the phospholipase of the present invention to screen for inhibiting compounds (see, for example, Reynolds L.J. et al., (1994) *Anal. Biochem.* 217: 25-32; Currie S. et al. (1994) *Biochem. J.* 304: 923-928; U.S. Patent No. 5,322,776; or U.S. Patent No. 6,440,683).

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In addition activity of foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous proteins against their physiological substrate(s) or derivatives thereof could be measured in cell-based or cell-free assays. Agents may also interfere with posttranslational modifications of a protein of the invention, such as phosphorylation and dephosphorylation, farnesylation,

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palmitoylation, acetylation, alkylation, ubiquitination, proteolytic processing, subcellular localization or degradation. Moreover, agents could influence the dimerization or oligomerization of a protein of the invention or, in a heterologous manner, of a protein of the invention with other proteins, for example, but not exclusively, docking proteins, enzymes, receptors, ion channels, uncoupling proteins, or translation factors. Agents could also act on the physical interaction of the proteins of this invention with other proteins, which are required for protein function, for example, but not exclusively, their downstream signaling.

Methods for determining protein-protein interaction are well known in the art. For example binding of a fluorescently labeled peptide derived from a protein of the invention to the interacting protein (or vice versa) could be detected by a change in polarisation. In case that both binding partners, which can be either the full length proteins as well as one binding partner as the full length protein and the other just represented as a peptide are fluorescently labeled, binding could be detected by fluorescence energy transfer (FRET) from one fluorophore to the other. In addition, a variety of commercially available assay principles suitable for detection of protein-protein interaction are well known in the art, for example but not exclusively AlphaScreen (PerkinElmer) or Scintillation Proximity Assays (SPA) by Amersham. Alternatively, the interaction of a protein of the invention with cellular proteins could be the basis for a cell-based screening assay, in which both proteins are fluorescently labeled and interaction of both proteins is detected by analysing cotranslocation of both proteins with a cellular imaging reader, as has been developed for example, but not exclusively, by Cellomics or EvotecOAI. In all cases the two or more binding partners can be different proteins with one being the protein of the invention, or in case of dimerization and/or oligomerization the protein of the invention itself. Proteins of the invention, for which one target mechanism of interest, but not the only one, would be such protein/protein interactions are foraging, CG5535, CG14217, Klp67A, CG10133,

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CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous proteins.

5 Assays for determining enzymatic, carrier, or ion channel activity of the proteins of the invention are well known in the art. Well known in the art are also a variety of assay formats to measure receptor-ligand binding.

10 Of particular interest are screening assays for agents that have a low toxicity for mammalian cells. The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of one or more of the proteins of the invention. Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than
15 about 2,500 Daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise carbocyclic or heterocyclic structures
20 and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups.

Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, nucleic acids and
25 derivatives, structural analogs or combinations thereof. Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and
30 oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and

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compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs. Where the screening
5 assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal.

Candidate agents may also be found in kinase assays where a kinase
10 substrate such as a protein, a peptide, a lipid, or an organic compound, which may or may not include modifications as further described below, or others are phosphorylated by the proteins or protein fragments of the invention. The kinase can be a protein of the invention (e.g. foraging or CG14217 homologous kinase) or a kinase, which is influenced in its
15 activity by a protein of the invention. A therapeutic candidate agent may be identified by its ability to increase or decrease the enzymatic activity of a protein of the invention. The kinase activity may be detected by change of the chemical, physical or immunological properties of the substrate due to phosphorylation. One example could be the transfer of radioisotopically
20 labelled phosphate groups from an appropriate donor molecule to the kinase substrate catalyzed by the polypeptides of the invention. The phosphorylation of the substrate may be followed by detection of the substrates autoradiography with techniques well known in the art.

25 Yet in another example, the change of mass of the substrate due to its phosphorylation may be detected by mass spectrometry techniques. One could also detect the phosphorylation status of a substrate with an analyte discriminating between the phosphorylated and unphosphorylated status of the substrate. Such an analyte may act by having different affinities for the phosphorylated and unphosphorylated forms of the substrate or by having
30 specific affinity for phosphate groups. Such an analyte could be, but is not limited to, an antibody or antibody derivative, a recombinant antibody-like

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structure, a protein, a nucleic acid, a molecule containing a complexed metal ion, an anion exchange chromatography matrix, an affinity chromatography matrix or any other molecule with phosphorylation dependend selectivity towards the substrate.

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Such an analyte could be employed to detect the kinase substrate, which is immobilized on a solid support during or after an enzymatic reaction. If the analyte is an antibody, its binding to the substrate could be detected by a variety of techniques as they are described in Harlow E. and Lane D.,
10 1988, *Antibodies: A Laboratory Manual*, CSH Lab Press, NY. If the analyte molecule is not an antibody, it may be detected by virtue of its chemical, physical or immunological properties, being endogenously associated with it or engineered to it.

15 Yet in another example the kinase substrate may have features, designed or endogenous, to facilitate its binding or detection in order to generate a signal that is suitable for the analysis of the substrates phosphorylation status. These features may be, but are not limited to, a biotin molecule or derivative thereof, a glutathione-S-transferase moiety, a moiety of six or
20 more consecutive histidine residues, an amino acid sequence or hapten to function as an epitope tag, a fluorochrome, an enzyme or enzyme fragment. The kinase substrate may be linked to these or other features with a molecular spacer arm to avoid steric hindrance.

25 In one example, the kinase substrate may be labelled with a fluorochrome. The binding of the analyte to the labelled substrate in solution may be followed by the technique of fluorescence polarization as it is described in the literature (see, for example, Deshpande S. et al., (1999) *Prog. Biomed. Optics* (SPIE) 3603: 261; Parker G.J. et al., (2000) *J. Biomol. Screen.* 5:
30 77-88; Wu P. et al., (1997) *Anal. Biochem.* 249: 29-36). In a variation of this example, a fluorescent tracer molecule may compete with the

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substrate for the analyte to detect kinase activity by a technique which is known to those skilled in the art as indirect fluorescence polarization.

Another technique for drug screening, which may be used, provides for high throughput screening of compounds having suitable binding affinity to the protein of interest as described in published PCT application WO84/03564. In this method, as applied to the proteins of the invention large numbers of different small test compounds are synthesized or provided on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with a protein of the invention or fragments thereof, and washed. Bound proteins are then detected by methods well known in the art. Purified proteins can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralising antibodies capable of binding a protein of the invention specifically compete with a test compound for binding the protein. In this manner, the antibodies can be used to detect the presence of any peptide, which shares one or more antigenic determinants with foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous protein.

The nucleic acids encoding a protein of the invention can be used to generate transgenic animals or site-specific gene modifications in cell lines. These transgenic non-human animals are useful in the study of the function and regulation of said protein in vivo. Transgenic animals, particularly mammalian transgenic animals, can serve as a model system for the investigation of many developmental and cellular processes common to humans. A variety of non-human models of metabolic disorders can be used to test modulators/effectors of the proteins of the invention.

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Misexpression (for example, overexpression or lack of expression) of a protein of the invention, particular feeding conditions, and/or administration of biologically active compounds can create models of metabolic disorders.

5 In one embodiment of the invention, such assays use mouse models of insulin resistance and/or diabetes, such as mice carrying gene knockouts in the leptin pathway (for example, ob (leptin) or db (leptin receptor) mice). Such mice develop typical symptoms of diabetes, show hepatic lipid accumulation and frequently have increased plasma lipid levels (see
10 Bruning J.C. et al, (1998) Mol. Cell. 2: 559-569). Susceptible wild type mice (for example C57Bl/6) show similar symptoms if fed a high fat diet. In addition to testing the expression of the proteins of the invention in such mouse strains (see Examples section), these mice could be used to test whether administration of a candidate modulator/effector alters for example
15 lipid accumulation in the liver, in plasma, or adipose tissues using standard assays well known in the art, such as FPLC, colorimetric assays, blood glucose level tests, insulin tolerance tests and others.

Transgenic animals may be made through homologous recombination in
20 non-human embryonic stem cells, where the normal locus of the gene encoding a protein of the invention is altered. Alternatively, a nucleic acid construct encoding a protein of the invention is injected into oocytes and is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, yeast artificial
25 chromosomes (YACs), and the like. The modified cells or animal are useful in the study of the function and regulation of the proteins of the invention. For example, a series of small deletions and/or substitutions may be made in the gene that encodes a protein of the invention to determine the role of particular domains of the protein, functions in pancreatic differentiation,
30 etc.

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Furthermore, variants of the genes of the invention like specific constructs of interest include anti-sense molecules, which will block the expression of the proteins of the invention, or expression of dominant negative mutations. A detectable marker, such as for example lac-Z or luciferase
5 may be introduced in the locus of a gene of the invention, where up regulation of expression of the genes of the invention will result in an easily detected change in phenotype.

One may also provide for expression of the genes of the invention or
10 variants thereof in cells or tissues where it is not normally expressed or at abnormal times of development. In addition, by providing expression of the proteins of the invention in cells in which they are not normally produced, one can induce changes in cell behavior.

15 DNA constructs for homologous recombination will comprise at least portions of the genes of the invention with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration do not need to contain regions of homology to mediate recombination. Conveniently, markers for positive
20 and negative selection are included. DNA constructs for random integration will consist of the nucleic acids encoding the proteins of the invention, a regulatory element (promoter), an intron and a poly-adenylation signal. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For non-human embryonic
25 stem (ES) cells, an ES cell line may be employed, or embryonic cells may be obtained freshly from a host, e.g. mouse, rat, guinea pig, etc. Such cells are grown on an appropriate fibroblast-feeder layer and are grown in the presence of leukemia inhibiting factor (LIF).

30 When non-human ES or embryonic cells or somatic pluripotent stem cells have been transfected, they may be used to produce transgenic animals. After transfection, the cells are plated onto a feeder layer in an appropriate

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medium. Cells containing the construct may be selected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be
5 used for embryo transfection and morula aggregation. Briefly, morulae are obtained from 4 to 6 week old superovulated females, the Zona Pellucida is removed and the morulae are put into small depressions of a tissue culture dish. The ES cells are trypsinized, and the modified cells are placed into the depression closely to the morulae. On the following day the
10 aggregates are transferred into the uterine horns of pseudopregnant females. Females are then allowed to go to term. Chimeric offsprings can be readily detected by a change in coat color and are subsequently screened for the transmission of the mutation into the next generation (F1-generation). Offspring of the F1-generation are screened for the
15 presence of the modified gene and males and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogenic or congenic grafts or transplants, or in vitro culture. The transgenic animals may be any non-human mammal, such as
20 laboratory animal, domestic animals, etc., for example, mouse, rat, guinea pig, sheep, cow, pig, and others. The transgenic animals may be used in functional studies, drug screening, and other applications and are useful in the study of the function and regulation of the proteins of the invention in vivo.

25 Finally, the invention also relates to a kit comprising at least one of

- (a) a nucleic acid molecule coding for a protein of the invention or a functional fragment thereof;
- (b) a protein of the invention or a functional fragment or an isoform thereof;
- (c) a vector comprising the nucleic acid of (a);
- (d) a host cell comprising the nucleic acid of (a) or the vector of (c);

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- (e) a polypeptide encoded by the nucleic acid of (a);
- (f) a fusion polypeptide encoded by the nucleic acid of (a);
- (g) an antibody, an aptamer or another modulator/effector of the nucleic acid of (a) or the polypeptide of (b), (e), or (f) and
- 5 (h) an anti-sense oligonucleotide of the nucleic acid of (a).

The kit may be used for diagnostic or therapeutic purposes or for screening applications as described above. The kit may further contain user instructions.

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The Figures show:

Figure 1 shows the triglyceride content of a *Drosophila* foraging (GadFly Accession Number CG10033) mutant. Shown is the change of triglyceride content of HD-EP(2)21996, HD-EP(2)22008, HD-EP(2)26800 flies caused by homozygous viable integration of the P-vector into the promoter/enhancer or into the cDNA of the foraging gene (referred to as 'HD-EP21996', column 2, 'HD-EP22008', column 3, 'HD-EP26800', column 4) in comparison to controls containing all flies of the EP collection (referred to as 'EP-control', column 1), and the change of triglyceride content of HD-EP(2)20702 flies caused by heterozygous viable integration of the P-vector into the promoter/enhancer or into an intron of the foraging gene (referred to as 'HD-EP20702/CyO', column 6) in comparison to controls containing all heterozygous flies of the EP collection containing a CyO balancer chromosome (referred to as 'EP-control CyO', column 5).

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Figure 2 shows the molecular organization of the mutated foraging gene locus.

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Figure 3 shows the BLASTP search result for the foraging gene product (Query) with the two best human homologous matches (Sbjct).

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Figure 4 shows the expression of the foraging homologs in mammalian (mouse) tissues.

Figure 4A shows the real-time PCR analysis of protein kinase, cGMP-dependent, type I (Prkg1) expression in wild-type mouse tissues.

5 Figure 4B shows the real-time PCR analysis of Prkg1 expression in different mouse models.

Figure 4C shows the real-time PCR analysis of Prkg1 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

10 Figure 4D shows the real-time PCR analysis of Prkg1 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 4E shows the real-time PCR analysis of protein kinase, cGMP-dependent, type II (Prkg2) expression in wild-type mouse tissues.

Figure 4F shows the real-time PCR analysis of Prkg2 expression in different mouse models.

15 Figure 4G shows the real-time PCR analysis of Prkg2 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

Figure 4H shows the real-time PCR analysis of Prkg2 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

20 Figure 5 shows the expression of a foraging homolog in mammalian (human) tissue. Shown is the quantitative analysis of protein kinase, cGMP-dependent, type II (PRKG2) expression in human abdominal adipocyte cells, during the differentiation from preadipocytes to mature adipocytes.

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Figure 6 shows the triglyceride content of a *Drosophila* cationic amino acid transporter (GadFly Accession Number CG5535) mutant. Shown is the change of triglyceride content of HD-EP(3)37469 flies caused by integration of the P-vector 5' of the cDNA (referred to as 'HD-EP37469', column 2), or by ectopic expression of the gene mainly in the fat body (referred to as 'HD-EP37469/FB', column 3) or mainly in the neurons

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(referred to as 'HD-EP37469/elav', column 4) of these flies in comparison to controls (referred to as 'EP-control', column 1).

5 Figure 7 shows the molecular organization of the mutated CG5535 (Gadfly Accession Number) gene locus.

Figure 8 shows the BLASTP search results for the CG5535 (Gadfly Accession Number) gene product (Query) with the three best human homologous matches (Sbjct).

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Figure 9 shows the expression of the CG5535 homologs in mammalian (mouse) tissues.

Figure 9A shows the real-time PCR analysis of solute carrier family 7, member 1 (Slc7a1) expression in wild-type mouse tissues.

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Figure 9B shows the real-time PCR analysis of Slc7a1 expression in different mouse models.

Figure 9C shows the real-time PCR analysis of Slc7a1 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

20 Figure 9D shows the real-time PCR analysis of Slc7a1 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 9E shows the real-time PCR analysis of solute carrier family 7, member 2 (Slc7a2) expression in wild-type mouse tissues.

25 Figure 9F shows the real-time PCR analysis of Slc7a2 expression in different mouse models.

Figure 9G shows the real-time PCR analysis of Slc7a2 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

30 Figure 9H shows the real-time PCR analysis of Slc7a2 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 9I shows the real-time PCR analysis of solute carrier family 7, member 3 (Slc7a3) expression in wild-type mouse tissues.

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Figure 9J shows the real-time PCR analysis of Slc7a3 expression in different mouse models.

Figure 9K shows the real-time PCR analysis of Slc7a3 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 10 shows the triglyceride content of a Drosophila CG14217 (GadFly Accession Number) mutant. Shown is the change of triglyceride content of HD-EP(X)10632 flies caused by integration of the P-vector into the annotated cDNA (referred to as 'HD-EP10632', column 2) in comparison to controls (referred to as 'EP-control', column 1).

Figure 11 shows the molecular organization of the mutated CG14217 (Gadfly Accession Number) gene locus.

Figure 12 shows the BLASTP search results for the CG14217 gene product (Query) with the four best human homologous matches (Sbjct).

Figure 13 shows the expression of CG14217 homologs in mammalian (mouse) tissues.

Figure 13A shows the real-time PCR analysis of RIKEN cDNA 1110033K02 gene (1110033K02Rik) expression in wild-type mouse tissues.

Figure 13B shows the real-time PCR analysis of 1110033K02Rik expression in different mouse models.

Figure 13C shows the real-time PCR analysis of 1110033K02Rik expression in mice fed with a high fat diet compared to mice fed with a standard diet.

Figure 13D shows the real-time PCR analysis of 1110033K02Rik expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

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Figure 13E shows the real-time PCR analysis of protein similar to thousand and one amino acid protein kinase (LOC192767) expression in wild-type mouse tissues.

5 Figure 13F shows the real-time PCR analysis of LOC192767 expression in different mouse models.

Figure 13G shows the real-time PCR analysis of LOC192767 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

10 Figure 13H shows the real-time PCR analysis of LOC192767 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 14 shows the triglyceride content of a Drosophila Kinesin-like protein at 67A (Klp67A; GadFly Accession Number CG10923) mutant. Shown is the change of triglyceride content of HD-EP(3)31624 flies caused by integration of the P-vector into the cDNA (referred to as 'HD-EP31624/TM3,Ser', column 2), or by ectopic expression of the Klp67A gene mainly in the fat body (referred to as 'HD-EP31624/FB', column 3) or mainly in the neurons (referred to as 'HD-EP31624/elav', column 4) of these flies in comparison to controls (referred to as 'EP-control', column 1).

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Figure 15 shows the molecular organization of the mutated Klp67A gene locus.

25 Figure 16 shows the expression of the Klp67A homolog in mammalian (mouse) tissues.

Figure 16A shows the real-time PCR analysis of kinesin family member 18A (Kif18a) expression in wild-type mouse tissues.

30 Figure 16B shows the real-time PCR analysis of Kif18a expression in different mouse models.

Figure 16C shows the real-time PCR analysis of Kif18a expression in mice fed with a high fat diet compared to mice fed with a standard diet.

Figure 16D shows the real-time PCR analysis of Kif18a expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

5 Figure 17 shows the triglyceride content of a *Drosophila* phospholipase A2 (GadFly Accession Number CG10133) mutant. Shown is the change of triglyceride content of HD-EP(3)31769 flies caused by integration of the P-vector into the cDNA (referred to as 'HD-EP31769', column 2), or by ectopic expression of the CG10133 gene mainly in the fat body (referred to
10 as 'HD-EP31769/FB', column 3) or mainly in the neurons (referred to as 'HD-EP31769/elav', column 4) of these flies in comparison to controls ('EP-control', column 1).

Figure 18 shows the molecular organization of the mutated CG10133 (Gadfly Accession Number) gene locus.
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Figure 19 shows the expression of a CG10133 homolog in mammalian (mouse) tissues.

Figure 19A shows the real-time PCR analysis of protein similar to phospholipase A2, group IVB (cytosolic) (LOC211429) expression in
20 wild-type mouse tissues.

Figure 19B shows the real-time PCR analysis of LOC211429 expression in different mouse models.

Figure 19C shows the real-time PCR analysis of LOC211429 expression
25 during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 20 shows triglyceride content of a *Drosophila* CG3967 (GadFly Accession Number) mutant. Shown is the change of triglyceride content of
30 HD-EP(3)37458 flies (referred to as 'HD-EP37458', column 2) caused by integration of the P-vector into the promoter of CG3967 (in comparison to controls (referred to as 'EP-control', column 1)).

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Figure 21 shows the molecular organization of the mutated CG3967 (Gadfly Accession Number) gene locus.

Figure 22 shows the triglyceride content of Drosophila CG32048 (GadFly Accession Number) mutants.

Figure 22A shows the change of triglyceride content of HD-EP(3)36547, HD-EP(3)37389, and HD-EP(3)36941 flies caused by integration of the P-vector into the promoter/enhancer region (referred to as 'HD-EP36547', column 2, 'HD-EP37389', column 3, 'HD-EP36941', column 4) in comparison to controls (referred to as 'EP-control', column 1).

Figure 22B shows the change of triglyceride content of HD-EP(3)30418 flies caused by integration of the P-vector into the promoter/enhancer region (referred to as 'HD-EP30418', column 2) in comparison to controls containing all flies of the EP collection (referred to as 'EP-control', column 1), and by ectopic expression of the CG32048 gene mainly in the fat body of these flies (referred to as 'HD-EP30418/FB', column 4) in comparison to controls containing all flies with the FB-Gal4 insertion (referred to as 'random EP/FB', column 3).

Figure 22C shows the change of triglyceride content of HD-EP(3)36364 flies caused by integration of the P-vector into the promoter/enhancer region (referred to as 'HD-EP36364', column 2) in comparison to controls (referred to as 'EP-control', column 1).

Figure 22D shows the change of triglyceride content of HD-EP(3)31171 flies caused by integration of the P-vector into the promoter/enhancer region (referred to as 'HD-EP31171', column 2), or by ectopic expression of the CG32048 gene mainly in the fat body (referred to as 'HD-EP31171/FB', column 3), or mainly in the neurons (referred to as 'HD-EP31171/elav', column 4) of these flies, in comparison to controls (referred to as 'EP-control', column 1).

Figure 23 shows the molecular organization of the mutated CG32048 (Gadfly Accession Number) gene locus.

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Figure 24 shows the triglyceride content of a *Drosophila* CG31605 (GadFly Accession Number) mutant. Shown is the change of triglyceride content of HD-EP(2)26044 flies caused by integration of the P-vector 9 base pairs 5' of the cDNA/ into the cDNA (referred to as 'HD-EP26044', column 2) in comparison to controls (referred to as 'EP-control', column 1).

Figure 25 shows the molecular organization of the mutated CG31605 (Gadfly Accession Number) gene locus.

Figure 26 shows the expression of the CG31605 homologs in mammalian (human) tissue.

Figure 26A shows the quantitative analysis of stromal cell derived factor receptor 1 (SDFR1) expression in human abdominal adipocyte cells, during the differentiation from preadipocytes to mature adipocytes.

Figure 26B shows the quantitative analysis of basigin (BSG) expression in human abdominal adipocyte cells, during the differentiation from preadipocytes to mature adipocytes.

Figure 27 shows the triglyceride content of a *Drosophila* Baldspot (GadFly Accession Number CG3971) mutant. Shown is the change of triglyceride content of HD-EP(3)37185 flies caused by integration of the P-vector into the promoter/enhancer of the cDNA (referred to as 'HD-EP37185', column 2), or by ectopic expression of the Baldspot gene mainly in the fat body (referred to as 'HD-EP37185/FB', column 3) or mainly in the neurons (referred to as 'HD-EP37185/elav', column 4) of these flies in comparison to controls ('EP-control', column 1).

Figure 28 shows the molecular organization of the mutated Baldspot gene locus.

Figure 29 shows the expression of the Baldspot homologs in mammalian (mouse) tissues.

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Figure 29A shows the real-time PCR analysis of ELOVL family member 6, elongation of long chain fatty acids (yeast) (Elov16) expression in wild-type mouse tissues.

5 Figure 29B shows the real-time PCR analysis of Elov16 expression in different mouse models.

Figure 29C shows the real-time PCR analysis of Elov16 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

10 Figure 29D shows the real-time PCR analysis of Elov16 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

Figure 29E shows the real-time PCR analysis of elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3 (Elov13) expression in wild-type mouse tissues.

15 Figure 29F shows the real-time PCR analysis of Elov13 expression in different mouse models.

Figure 29G shows the real-time PCR analysis of Elov13 expression in mice fed with a high fat diet compared to mice fed with a standard diet.

20 Figure 29H shows the real-time PCR analysis of Elov13 expression during the differentiation of 3T3-L1 cells from preadipocytes to mature adipocytes.

25 Figure 30 shows the expression of a Baldspot homolog in mammalian (human) tissue. Shown is the quantitative analysis of ELOVL family member 6, elongation of long chain fatty acids (ELOVL6) expression in human abdominal adipocyte cells, during the differentiation from preadipocytes to mature adipocytes.

30 Figure 31 shows the triglyceride content of a Drosophila CG13625 (GadFly Accession Number) mutant. Shown is the change of triglyceride content of HD-EP(3)37410 flies caused by integration of the P-vector 5' of the cDNA (referred to as 'HD-EP37410', column 2), or by ectopic expression of the CG13625 gene mainly in the fat body (referred to as 'HD-EP37410/FB',

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column 3) or mainly in the neurons (referred to as 'HD-EP37410/elav', column 4) of these flies in comparison to controls (referred to as 'EP-control', column 1).

5 Figure 32 shows the molecular organization of the mutated CG13625 (Gadfly Accession Number) gene locus.

Figure 33 shows the triglyceride content of a *Drosophila fusilli* (GadFly Accession Number CG8205) mutant. Shown is the change of triglyceride
10 content of HD-EP(2)25801 flies caused by integration of the P-vector 5' of/into the cDNA (referred to as 'HD-EP25801', column 2), or by ectopic expression of the fusilli gene mainly in the fat body (referred to as 'HD-EP25801/FB', column 3) or mainly in the neurons of these flies (referred to as 'HD-EP25801/elav', column 4) in comparison to controls
15 (referred to as 'EP-control', column 1).

Figure 34 shows the molecular organization of the mutated fusilli gene locus.

20 Figure 35 shows the triglyceride content of a *Drosophila schnurri* (shn; GadFly Accession Number CG7734) mutant. Shown is the change of triglyceride content of HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, and HD-EP(2)26808 flies caused by integration of the P-vector 5' of the cDNA /into an intron of schnurri (referred to as 'HD-EP21919', column 2,
25 'HD-EP22018', column 3, 'HD-EP26878/CyO', column 4, and 'HD-EP26808', column 5) in comparison to controls ('EP-control', column 1).

Figure 36 shows the molecular organization of the mutated schnurri gene
30 locus.

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Figure 37 shows the expression of a schnurri homolog in mammalian (human) tissue. Shown is the quantitative analysis of human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1) expression in human abdominal adipocyte cells, during the differentiation
5 from preadipocytes to mature adipocytes.

The examples illustrate the invention:

10 Example 1: Measurement of triglyceride content in *Drosophila*

Mutant flies are obtained from proprietary or publicly available fly mutation stock collections. The flies are grown under standard conditions known to those skilled in the art. In the course of the experiment, additional feedings
15 with bakers yeast (*Saccharomyces cerevisiae*) are provided. The average change of triglyceride content of *Drosophila* containing the EP-vectors in homozygous viable, hemizygous viable, or heterozygous viable/homozygous lethal integration, was investigated in comparison to control flies (see Figures 1, 6, 10, 14, 17, 20, 22, 24, 27, 31, 33, and
20 35). Further, the change of triglyceride content of *Drosophila* containing the the EP-vectors in combination of with tissue specific Gal4-drivers that cause tissue specific ectopic expression (fat body and neurons) were investigated in comparison to control flies. The offspring of males containing the EP-vectors that are crossed to FB-Gal4 virgins carries a copy
25 of the EP-vector and a copy of the FB-Gal4 vector, leading to ectopic expression of adjacent genomic DNA sequences 3' of the EP-vector integration locus, mainly in the fat body of these flies. The offspring of males containing the EP-vectors that are crossed to elav-Gal4 virgins carries a copy of the EP-vector and a copy of the elav-Gal4 vector, leading
30 to ectopic expression of adjacent genomic DNA sequences 3' of the EP-vector integration locus, mainly in neurons of these flies. For determination of triglyceride, flies were incubated for 5 min at 90°C in an

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aqueous buffer using a waterbath, followed by hot extraction. After another 5 min incubation at 90°C and mild centrifugation, the triglyceride content of the flies extract was determined using Sigma Triglyceride (INT 336-10 or -20) assay by measuring changes in the optical density according to the manufacturer's protocol. As a reference the protein content of the same extract was measured using BIO-RAD DC Protein Assay according to the manufacturer's protocol. The assays were repeated several times.

The average triglyceride level of all flies of the EP collection (referred to as 'EP-control') is shown as 100% in the first columns in Figures 1, 6, 10, 14, 17, 20, 22 A-D, 24, 27, 31, 33, and 35. The average triglyceride level of all heterozygous flies of the EP collection containing a CyO balancer chromosome (referred to as 'EP-control CyO') is shown as 100% in column 5 in Figure 1. The average triglyceride level of all flies containing the FB-Gal4 vector (referred to as 'random EP/FB') is shown as 100% in column 3 in Figure 22B. Standard deviations of the measurements are shown as thin bars.

HD-EP(2)21996 homozygous flies (column 2 in Figure 1, 'HD-EP21996') and HD-EP(2)22008 homozygous flies (column 3 in Figure 1, 'HD-EP22008') show constantly a lower triglyceride content, and HD-EP(2)26800 homozygous flies (column 4 in Figure 1, 'HD-EP26800') show constantly a higher triglyceride content than the controls. HD-EP(2)20702 heterozygous flies (column 6 in Figure 1, 'HD-EP20702/CyO') show constantly a higher triglyceride content than the heterozygous controls.

HD-EP(3)37469 homozygous flies (column 2 in Figure 6, 'HD-EP37469') show a slightly higher triglyceride content than the controls. The offspring of HD-EP(3)37469 males that are crossed to FB-Gal4 virgins (column 3 in Figure 6, 'HD-EP37469/FB'), shows constantly a slightly lower triglyceride

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content than the controls. Both values are in the normal range compared to controls and therefore represent no significant change of triglyceride content. The offspring of HD-EP(3)37469 males that are crossed to elav-Gal4 virgins (column 4 in Figure 6, 'HD-EP37469/elav') shows
5 constantly a lower triglyceride content than the controls.

HD-EP(X)10632 hemizygous flies show constantly a higher triglyceride content than the controls (column 2 in Figure 10, 'HD-EP10632').

10 HD-EP(3)31624 heterozygous flies (column 2 in Figure 14, 'HD-EP31624/TM3,Ser') show a slightly lower triglyceride content than the controls. The offspring of HD-EP(3)31624 males that are crossed to FB-Gal4 virgins (column 3 in Figure 14, 'HD-EP31624/FB') shows constantly a slightly higher triglyceride content than the controls. Both
15 values are in the normal range compared to controls and therefore represent no significant change of triglyceride content. The offspring of HD-EP(3)31624 males that are crossed to elav-Gal4 virgins (column 4 in Figure 14, 'HD-EP31624/elav') shows constantly a higher triglyceride content than the controls.

20 HD-EP(3)31769 homozygous flies (column 2 in Figure 17, 'HD-EP31769') show constantly a slightly lower triglyceride content than the controls. The offspring of HD-EP(3)31769 males that are crossed to FB-Gal4 virgins (column 3 in Figure 17, 'HD-EP31769/FB') shows constantly a slightly
25 higher triglyceride content than the controls. Both values are in the normal range compared to controls and therefore represent no significant change of triglyceride content. The offspring of HD-EP(3)31769 males that are crossed to elav-Gal4 virgins (column 4 in Figure 17, 'HD-EP31769/elav') shows constantly a lower triglyceride content than the controls.

30 HD-EP(3)37458 homozygous flies show constantly a higher triglyceride content than the controls (column 2 in Figure 20, 'HD-EP37458').

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HD-EP(3)36547 homozygous flies (column 2 in Figure 22A, 'HD-EP36547') and HD-EP(3)37389 homozygous flies (column 3 in Figure 22A, 'HD-EP37389') show constantly a lower triglyceride content than the controls. HD-EP(3)36941 homozygous flies (column 4 in Figure 22A, 'HD-EP36941') show constantly a higher triglyceride content than the controls.

HD-EP(3)30418 homozygous flies (column 2 in Figure 22B, 'HD-EP30418') show constantly a higher triglyceride content than the controls. The offspring of HD-EP(3)30418 males that are crossed to FB-Gal4 virgins (column 4 in Figure 22B, 'HD-EP30418/FB') shows constantly a higher triglyceride content than the controls.

HD-EP(3)36364 homozygous flies (column 2 in Figure 22C, 'HD-EP36547') show constantly a higher triglyceride content than the controls.

HD-EP(3)31171 homozygous flies (column 2 in Figure 22D, 'HD-EP31171') show constantly a slightly lower triglyceride content than the controls. This value is in the normal range compared to controls and therefore represents no significant change of triglyceride content. The offspring of HD-EP(3)31171 males that are crossed to FB-Gal4 virgins (column 3 in Figure 22D, 'HD-EP31171/FB') shows constantly a higher triglyceride content than the controls. The offspring of HD-EP(3)31171 males that are crossed to elav-Gal4 virgins (column 4 in Figure 22D, 'HD-EP31171/elav') shows constantly a higher triglyceride content than the controls.

HD-EP(2)26044 homozygous flies (column 2 in Figure 24, 'HD-EP26044') show constantly a lower triglyceride content than the controls.

HD-EP(3)37185 homozygous flies (column 2 in Figure 27, 'HD-EP37185') show constantly a lower triglyceride content than the controls. The offspring of HD-EP(3)37185 males that are crossed to FB-Gal4 virgins

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(column 3 in Figure 27, 'HD-EP37185/FB') shows constantly a slightly lower triglyceride content than the controls. This value is in the normal range compared to controls and therefore represents no significant change of triglyceride content. The offspring of HD-EP(3)37185 males that are
5 crossed to elav-Gal4 virgins (column 4 in Figure 27, 'HD-EP37185/elav') shows constantly a higher triglyceride content than the controls.

Example 2: Identification of Drosophila genes associated with triglyceride
10 metabolism

Nucleic acids encoding the proteins of the present invention were identified using a plasmid-rescue technique. Genomic DNA sequences were isolated that are localized adjacent to the EP vector (herein HD-EP(2)21996,
15 HD-EP(2)22008, HD-EP(2)26800, HD-EP(2)20702, HD-EP(3)37469, HD-EP(X)10632, HD-EP(3)31624, HD-EP(3)31769, HD-EP(3)37458, HD-EP(3)31600, HD-EP(3)36681, HD-EP(3)36956, HD-EP(3)36547, HD-EP(3)37389, HD-EP(3)36941, HD-EP(3)30418, HD-EP(3)36364, HD-EP(3)31171, HD-EP(2)26044, HD-EP(3)37185, HD-EP(3)37410,
20 HD-EP(2)25801, HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, or HD-EP(2)26808) integration. Using those isolated genomic sequences public databases like Berkeley Drosophila Genome Project (GadFly) were screened, thereby identifying the integration sites of the vectors, and the corresponding genes. The molecular organization of these gene loci is
25 shown in Figures 2, 7, 11, 15, 18, 21, 23, 25, 28, 32, 34, and 36.

In the upper half of Figure 2 and in the lower half of Figures 21, 23, 25, 34, and 36, genomic DNA sequence is represented by the assembly as a black scaled double-headed arrow in the middle that includes the
30 integration sites of the vectors for lines HD-EP(2)21996, HD-EP(2)22008, HD-EP(2)26800, HD-EP(2)20702, HD-EP(3)37458, HD-EP(3)31600, HD-EP(3)36681, HD-EP(3)36956, HD-EP(3)36547, HD-EP(3)37389,

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HD-EP(3)36941, HD-EP(3)30418, HD-EP(3)36364, HD-EP(3)31171, HD-EP(2)26044, HD-EP(2)25801, HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, or HD-EP(2)26808. Ticks represent the coordinates of the genomic DNA (1000 base pairs (Figure 21) or 10000 base pairs (Figures 2, 23, 25, 34, and 36) per tick). The grey arrows in the upper part of the figures represent BAC clones, the black arrows in the topmost part of the figures represent the sections of the chromosomes. The insertion sites of the P-elements in the *Drosophila* lines are shown as triangles and are labeled. The cDNA sequences of the predicted genes (as predicted by the Berkeley *Drosophila* Genome Project, GadFly and by Magpie) are shown as dark grey bars (exons), linked by dark grey lines (introns), and are labeled (see also key at the bottom of the figures).

In Figures 7, 11, 15, 18, 28, and 32, and in the upper half of Figures 21, 23, 25, 34, and 36, genomic DNA sequence is represented by the assembly as a dotted black line in the middle that includes the integration sites of the vectors for lines HD-EP(3)37469, HD-EP(X)10632, HD-EP(3)31624, HD-EP(3)31769, HD-EP(3)37458, HD-EP(3)31600, HD-EP(3)36681, HD-EP(3)36956, HD-EP(3)36547, HD-EP(3)37389, HD-EP(3)36941, HD-EP(3)30418, HD-EP(3)36364, HD-EP(3)31171, HD-EP(2)26044, HD-EP(3)37185, HD-EP(3)37410, HD-EP(2)25801, HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, or HD-EP(2)26808. Numbers represent the coordinates of the genomic DNA. The upper parts of the figures represent the sense strand "+", the lower parts represent the antisense strand "-". The insertion sites of the P-elements in the *Drosophila* lines are shown as triangles or boxes in the "P-elements +" and/or "P-elements -" lines. Transcribed DNA sequences (ESTs) are shown as grey bars in the "EST +", "EST -", "IPI +", and/or the "IPI -" lines, and predicted cDNAs are shown as bars in the "cDNA +" and/or "cDNA -" lines. Predicted exons of the cDNAs are shown as dark grey bars and predicted introns are shown as light grey bars.

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The HD-EP(2)21996, HD-EP(2)22008, and HD-EP(2)26800 vectors are homozygous viable integrated 80 base pairs 5' / into an intron of the cDNA of a Drosophila gene, and the HD-EP(2)20702 vector is homozygous lethal/heterozygous viable integrated 6243 base pairs 5' / into an intron of the cDNA of a Drosophila gene in sense orientation, identified as foraging (for, GadFly Accession Number CG10033). The chromosomal localization site of integrations of the vectors of HD-EP(2)21996, HD-EP(2)22008, HD-EP(2)26800, and HD-EP(2)20702 are at gene locus 2L, 24A2-4. In the upper part of Figure 2, the molecular organization of the foraging gene locus, as annotated by GadFly (release 3), is shown. The insertion sites of the P-elements in Drosophila HD-EP(2)21996, HD-EP(2)22008, HD-EP(2)26800, and HD-EP(2)20702 lines are labeled. The alternative splice variants of gene foraging (referred to as 'for') are labeled. In the lower part of Figure 2, the molecular organization of the foraging gene locus, as annotated by FlyBase, is shown. The splice variants of the foraging gene show different 5' ends.

The HD-EP(3)37469 vector is homozygous viable integrated about 4500 base pairs 5' of a Drosophila gene in sense orientation, identified as CG5535 (GadFly Accession Number; GenBank Accession Number AY118300 for the cDNA, AAM48329, AAF49291, and AAF49292 for the protein). The chromosomal localization site of integration of the vector of HD-EP(3)37469 is at gene locus 3L, 75A4. In Figure 7, the coordinates of the genomic DNA start at position 17748000 on chromosome 3L, ending at position 17759500. The insertion site of the P-element in Drosophila HD-EP(3)37469 line is shown in the "P Elements -" line and is labeled. The predicted cDNA of the CG5535 gene shown in the "cDNA -" line is labeled, the corresponding ESTs are shown in the "EST -" and the "IPI -" lines.

The HD-EP(X)10632 vector is hemizygous viable integrated into the cDNA (base pair 188) of a Drosophila gene in antisense orientation, identified as

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CG14217 (GadFly Accession Number). The chromosomal localization site of integration of the vector of HD-EP(X)10632 is at gene locus X, 18D3. In Figure 11, the coordinates of the genomic DNA start at position 19307388 on chromosome X, ending at position 19319888. The insertion site of the P-element in Drosophila HD-EP(X)10632 line is shown in the "P Elements + " line and is labeled. The gene CG14217 shown in the "cDNA -" line is labeled, the corresponding ESTs are shown in the "EST -" and the "IPI -" lines.

The HD-EP(3)31624 vector is homozygous lethal/heterozygous viable integrated into the cDNA (base pair 25; 5' of the translation start) of a Drosophila gene in sense orientation, identified as Klp67A (GadFly Accession Number CG10923, GenBank Accession Number NM_079268 for the cDNA, NP_523992 for the protein). The chromosomal localization site of integration of the vector of HD-EP(3)31624 is at gene locus 3L, 67A9-B1. In Figure 15, the coordinates of the genomic DNA start at position 9282080 on chromosome 3L, ending at position 9288330. The insertion site of the P-element in Drosophila HD-EP(3)31624 line is shown in the "P Elements -" line and is labeled. The gene Klp67A shown in the "cDNA -" line is labeled, the corresponding ESTs are shown in the "EST -" and the "IPI -" lines.

The HD-EP(3)31769 vector is homozygous viable integrated into the cDNA (base pair 91; 5' of the translation start) of a Drosophila gene in sense orientation, identified as CG10133 (GadFly Accession Number, GenBank Accession Numbers AY122113 for the cDNA, AAM52625, and AAF49813 for the protein). The chromosomal localization site of the integration of the vectors of HD-EP(3)31769 is at gene locus 70B1 on chromosome 3L. In Figure 18, the coordinates of the genomic DNA start at position 13363718 on chromosome 3L, ending at position 13365281. The insertion site of the P-element in Drosophila HD-EP(3)31769 line is shown in the "P Elements -" line and is labeled. The gene CG10133 is shown in the "cDNA -" line, the

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corresponding ESTs are shown in the "EST -" and the "IPI -" lines and are labeled.

5 The HD-EP(3)37458 vector is homozygous viable integrated into the promoter (5' of EST clone HL01728) of a Drosophila gene in sense orientation, identified as CG3967 (GadFly Accession Number). The chromosomal localization site of integration of the vector of HD-EP(3)37458 is at gene locus 3L, 67B2-3. In the upper part of Figure 21, the coordinates of the genomic DNA start at position 9331000 on chromosome 3L, ending at position 9349000. The insertion site of the P-element in Drosophila HD-EP(3)37458 line is shown in the "P Elements -" line and is labeled. The gene CG3967 is shown in the "cDNA -" line and is labeled, the corresponding ESTs are shown in the "EST -" line and are labeled. In the lower part of Figure 21 the molecular organization of the CG3967 gene, as annotated by GadFly (release 3), is shown. The splice variants of CG3967 show different 5' ends of the gene resulting in an integration site of the HD-EP(3)37458 1 base pair 5' of the cDNA of three CG3967 splice variants. Additionally the insertion sites of the P-elements in Drosophila HD-EP(3)31600, HD-EP(3)36681 and HD-EP(3)36959 lines are are labeled.

25 The HD-EP(3)36941 and HD-EP(3)31171 vectors are homozygous viable integrated in the promoter/ enhancer region of a Drosophila gene in sense orientation, and the HD-EP(3)36547, HD-EP(3)37389, HD-EP(3)30418, and HD-EP(3)36364 vectors are homozygous viable integrated into the promoter/enhancer region of a Drosophila gene in antisense orientation, identified as CG32048 (GadFly Accession Number). The chromosomal localization site of integration of the vectors is at gene locus 3L, 67B12-C1. In the upper part of Figure 23, the coordinates of the genomic DNA start at position 9460000 on chromosome 3L, ending at position 9517000. The insertion site of the P-elements in Drosophila HD-EP(3)36941, and HD-EP(3)31171 lines is shown in the "P Elements +"

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line and is labeled, and the insertion site of the P-elements in Drosophila HD-EP(3)36547, HD-EP(3)37389, HD-EP(3)30418, and HD-EP(3)36364 lines is shown in the "P Elements -" line and is labeled. The gene CG32048 is shown in the "cDNA +" line, the corresponding ESTs are shown in the "EST +" and the "IPI +" lines and are labeled. In the lower part of Figure 23, the molecular organization of the CG32048 gene locus, as annotated by GadFly (release 3), is shown. The insertion sites of the P-elements in the Drosophila lines are labeled. The splice variants show different 5' ends of the CG32048 gene.

The HD-EP(2)26044 vector is homozygous viable integrated into the promoter of a Drosophila gene in sense orientation, identified as CG31605 (GadFly Accession Number; also referred to as 'dmel2L_DKmel2L_DK12'). The chromosomal localization site of integration of the vector of HD-EP(2)26044 is at gene locus 2L, 28E3-5. In the upper part of Figure 25, the coordinates of the genomic DNA start at position 7984000 on chromosome 2L, ending at position 8019000. The insertion site of the P-element in Drosophila HD-EP(2)26044 line is shown in the "P Elements +" line and is labeled. The gene CG31605 (referred to as 'dmel2L_DKdmel2L_DK12') shown in the "cDNA +" line is labeled, the corresponding ESTs are shown in the "EST +" line. In the lower part of Figure 25 the molecular organization of the CG31605 gene, as annotated by GadFly (release 3), is shown. The insertion site of the P-element in Drosophila HD-EP(2)26044 line is labeled. The splice variants show different 5' ends of the gene resulting in an integration site of the vector of HD-EP(2)26044 into the promoter (9 base pairs 5') of four CG31605 splice variants) and into the first intron of four other CG31605 splice variants.

The HD-EP(3)37185 vector is homozygous viable integrated 14 base pairs 5' of a Drosophila gene in sense orientation, identified as Baldspot (GadFly Accession Number CG3971, GenBank Accession Numbers NM_140652 for the cDNA, MP_648909 and AAF49430 for the protein). The chromosomal

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localization site of the integration of the vector of HD-EP(3)37185 is at gene locus 73B4-5 on chromosome 3L. In Figure 28, the coordinates of the genomic DNA start at position 16547000 on chromosome 3L, ending at position 16562000. The insertion site of the P-element in Drosophila HD-EP(3)37185 line is shown in the "P Elements -" line and is labeled. 5
Magpie). The gene Baldspot is shown in the "cDNA -" line, the corresponding ESTs are shown in the "EST -" and "IPI -" lines and are labeled.

10 The HD-EP(3)37410 vector is homozygous viable integrated about 3500 base pairs 5' of a Drosophila gene in sense orientation, identified as CG13625 (GadFly Accession Number; GenBank Accession Numbers NM_143015 for the cDNA, NP_651272 for the protein). The chromosomal localization site of the integration of the vector of HD-EP(3)37410 is at 15
gene locus 3R, 96A9. In Figure 32, the coordinates of the genomic DNA start at position 20314000 on chromosome 3R, ending at position 20324000. The insertion site of the P-element in Drosophila HD-EP(3)37410 line is shown in the "P Elements -" line and is labeled. The gene CG13625 is shown in the "cDNA -" line, the corresponding ESTs are 20
shown in the "EST -" and "IPI -" lines and are labeled.

The HD-EP(2)25801 vector is homozygous viable integrated into the cDNA (base pair 21; 5' of the translation start) of a Drosophila gene in sense orientation, identified as fusilli (fus; GadFly Accession Number CG8205; 25
GenBank Accession Number NM_079952 for the cDNA, NP_524691 for the protein). The chromosomal localization site of integration of the vector of HD-EP(2)25801 is at gene locus 2R, 52C3-4. In the upper part of Figure 34, the coordinates of the genomic DNA start at position 10618000 on chromosome 2R, ending at position 10648000. The insertion site of the 30
P-element in Drosophila HD-EP(2)25801 line is shown in the "P Elements -" line and is labeled. The gene fusilli is shown in the "cDNA -" line, the corresponding ESTs are shown in the "EST -" and "IPI -" lines and are

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labeled. In the lower part of Figure 34 the molecular organization of the fusilli gene (referred to as 'fus'), as annotated by GadFly (release 3), is shown. The splice variants show different 5' ends of the gene resulting in an integration site of the HD-EP(2)25801 into the cDNA CG8205-RE but
5 very likely affecting also three other fusilli splice variants.

The HD-EP(2)21919 and HD-EP(2)26808 vectors are homozygous viable integrated into the promoter, the HD-EP(2)22018 vector is homozygous viable integrated into an of a Drosophila gene in sense orientation, and the
10 HD-EP(2)26878 vector is homozygous lethal/heterozygous viable integrated into the promoter or an intron of a Drosophila gene in antisense orientation, identified as schnurri (shn; GadFly Accession Number CG7734). The chromosomal localization sites of integration of the vectors of HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, and HD-EP(2)26808 are
15 at gene locus 2R, 47E1. In the upper part of Figure 36, the coordinates of the genomic DNA start at position 6170000 on chromosome 2R, ending at position 6222000. The insertion sites of the P-elements in Drosophila HD-EP(2)21919, HD-EP(2)26808, and HD-EP(2)22018 line are shown in the "P Elements +" line and are labeled, and the insertion site of the
20 P-element in Drosophila HD-EP(2)26878 line is shown in the "P Elements -" line and is labeled. The gene schnurri is shown in the "cDNA +" line, the corresponding ESTs are shown in the "EST +" and "IPI +" lines and are labeled. In the lower part of Figure 36, the molecular organization of the schnurri gene, as annotated by GadFly (release 3), is shown. The insertion
25 sites of the P-elements in Drosophila HD-EP(2)21919, HD-EP(2)22018, HD-EP(2)26878, or HD-EP(2)26808 lines are labeled. The splice variants show different 5' ends of the gene resulting in integration sites of the vectors of HD-EP(2)22018 and HD-EP(2)26878 into the promoter of one schnurri splice variant and into an intron of two other schnurri splice
30 variants.

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Expression of the genes described above could be effected by integration of the vectors into the transcription units, leading to a change in the amount of the energy storage triglycerides.

5

Example 3: Identification of human homologous genes and proteins

The Drosophila genes and proteins encoded thereby with functions in the regulation of triglyceride metabolism were further analysed using the
10 BLAST algorithm searching in publicly available sequence databases and mammalian homologs were identified (see Table 1 and Figures 3, 8, and 12).

The term "polynucleotide comprising the nucleotide sequence as shown in
15 GenBank Accession number" relates to the expressible gene of the nucleotide sequences deposited under the corresponding GenBank Accession number. The term "GenBank Accession number" relates to NCBI GenBank database entries (Ref.: Benson et al., Nucleic Acids Res. 28 (2000) 15-18). Sequences homologous to Drosophila foraging, CG5535,
20 CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri were identified using the publicly available program BLASTP 2.2.3 of the non-redundant protein data base of the National Center for Biotechnology Information (NCBI) (see, Altschul S.F. et al., (1997) Nucleic Acids Res. 25: 3389-3402).

25

Table 1: Human homologs of the Drosophila (Dm) genes

Dm gene	Homo sapiens homologous genes and proteins		
Acc. No.	Accession Number		Name
Name	cDNA	Protein	
CG10033 <i>foraging</i>	NM_006258	NP_006249	protein kinase, cGMP-dependent, type I (PRKG1)
	NM_006259	NP_006250	protein kinase, cGMP-dependent, type II (PRKG2)
CG5535	NM_003045	NP_003036	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1 (SLC7A1)
	NM_003046	NP_003037	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 2 (SLC7A2)
	NM_032803	NP_116192	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 3 (SLC7A3)
CG14217	NM_016281	NP_057365	STE20-like kinase (JIK)
	XM_290796	XP_290796	KIAA1361 protein (KIAA1361)
	NM_004783	NP_004774	thousand and one amino acid protein kinase (TAO1)
	NM_016151	NP_057365	prostate derived STE20-like kinase PSK (PSK)
CG10923 <i>Klp67A</i>	NM_031217	NP_112494	kinesin family member 18A (DKFZP434G2226)
	NM_153209	NP_694941	hypothetical protein FLJ37300 (FLJ37300)
CG10133	NM_005090	NP_005081	phospholipase A2, group IVB (cytosolic) (PLA2G4B)
CG3967	NM_024909	NP_079185	chromosome 6 open reading frame 134 (C6orf134); hypothetical protein FLJ13158
	ENSG00000180255	ENSP00000319549	hypothetical protein FLJ13158
CG32048	NM_014697	NP_055512	C-terminal PDZ domain ligand of neuronal nitric oxide synthase (CAPON)
CG31605	NM_012428	NP_036560	stromal cell derived factor receptor 1 (SDFR1), transcript variant beta / isoform b
	NM_017455	NP_059429	stromal cell derived factor receptor 1 (SDFR1), transcript variant alpha / isoform a
	NM_001728	NP_001719	basigin (OK blood group) (BSG)
	AF548371	AAN40694	basigin long isoform (BSG)

5	CG3971 <i>Baldspot</i>	NM_024090	NP_076995	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast) (ELOVL6)
		NM_152310	NP_689523	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3 (ELOVL3)
	CG13625	NM_032725	NP_116114	hypothetical protein MGC13125 (MGC13125)
	CG8205 <i>fusilli</i>	NM_024939	NP_079215	hypothetical protein FLJ21918 (FLJ21918)
		NM_017697	NP_060167	hypothetical protein FLJ20171 (FLJ20171)
10	CG7734 <i>schnurri</i>	NM_002114	NP_002105	human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1)
		NM_006734	NP_006725	human immunodeficiency virus type I enhancer binding protein 2 (HIVEP2)
		NM_024503	NP_078779	human immunodeficiency virus type I enhancer binding protein 3 (HIVEP3)
		XM_066058	XP_066058	chromosome 20 open reading frame 174 (C20orf174)

15

foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous proteins and nucleic acid molecules coding therefore are obtainable from insect or vertebrate species, e.g. mammals or birds. Particularly preferred are nucleic acids as described in Table 1.

As shown in Figure 3, gene product of *Drosophila* foraging (GadFly Accession Number CG10033, GenBank Accession Number NM_058139 for the cDNA, NP_477487 for the protein) is 74% homologous to human protein kinase, cGMP-dependent, regulatory, type 1, beta isozym (GenBank Accession Number NP_006249.1 for the protein, NM_006258 for the cDNA) and 63% homologous to human protein kinase, cGMP-dependent, type II (GenBank Accession Number NP_006250.1 for the protein, NM_006259 for the cDNA). Additionally, gene product of *Drosophila* foraging is 74% homologous to human cGMP-dependent protein kinase 1, alpha isozym (GenBank Accession Number Q13976 for the protein),

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81%/38% homologous to human protein kinase, cGMP-dependent, type 1 (GenBank Accession Number XP_165548.1 for the protein, XM_165548 for the cDNA), 70%/55% homologous to human protein kinase, cGMP-dependent, type II (GenBank Accession Number XP_113447.1 for the protein, XM_113447 for the cDNA), and 63% homologous to human cGMP-dependant protein kinase (GenBank Accession Number CAA76073.1 for the protein, Y16106 for the cDNA). *Drosophila* foraging also shows homology on protein level to mouse protein kinase, cGMP-dependent, type 1 (GenBank Accession Number NM_011160 for the cDNA), and type II (GenBank Accession Number NM_008926 for the cDNA).

As shown in Figure 8, gene product of *Drosophila* CG5535 (GadFly Accession Number; GenBank Accession Number AY118300 for the cDNA, AAM48329, AAF49291, and AAF49292 for the protein) is 64% homologous to human solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 (GenBank Accession Number NP_003037.1 for the protein, NM_003046 for the cDNA), 64% homologous to human solute carrier family 7 (cationic amino acid transporter, y+ system), member 1 (GenBank Accession Number NP_003036.1 for the protein, NM_003045 for the cDNA), and 64% homologous to human solute carrier family 7 (cationic amino acid transporter, y+ system), member 3 (GenBank Accession Number NP_116192.2 for the protein, NM_032803 for the cDNA). *Drosophila* CG5535 also shows homology on protein level to mouse solute carrier family 7 (cationic amino acid transporter, y+ system), members 1, 2, and 3 (GenBank Accession Numbers NM_007513, NM_007514, and NM_007515, respectively).

As shown in Figure 12, gene product of *Drosophila* CG14217 (GadFly Accession Number, GenBank Accession Number NM_134475 for the cDNA, NP_608319 and AAF48973 for the protein) is 84%/56%/43/41% homologous to human prostate derived STE20-like kinase PSK (GenBank

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Accession Number NP_057235 for the protein, NM_016151 for the cDNA),
73%/66% homologous to human KIAA1361 protein (GenBank Accession
Number BAA92599.1 for the protein, AB037782 for the cDNA), 71%/65%
homologous to human STE20-like kinase (GenBank Accession Number
5 NP_057365.2 for the protein, NM_016281 for the cDNA), and 84%
homologous to human thousand and one amino acid protein kinase
(GenBank Accession Number NP_004774.1 for the protein, NM_004783
for the cDNA). Additionally, gene product of Drosophila CG14217 is
73%/66% homologous to human serin/threonin kinase TAO1 (GenBank
10 Accession Number AAL12217.1 for the protein, AY049015 for the cDNA),
72%/66% homologous to human STE20-like kinase (GenBank Accession
Number AAG38502.1 for the protein, AF263312 for the cDNA), 70%/65%
homologous to human STE20-like kinase (GenBank Accession Number
AAF14559.1 for the protein, AF179867 for the cDNA), 71%/65%
15 homologous to human protein similar to serine kinase (JIK; GenBank
Accession Number XP_045006.1 for the protein, XM_045006 for the
cDNA), 71%/65% homologous to human STE20-like kinase (GenBank
Accession Number AAG38501.1 for the protein, AF263311 for the cDNA),
and 66%/69% homologous to human protein similar to serine/threonine
20 kinase TAO1 (KIAA1361; GenBank Accession Number XP_030845.7 for
the protein, XM_030845 for the cDNA). Drosophila CG14217 also shows
homology on protein level to mouse proteins (GenBank Accession Number
XM_126386, XM_109632, and XM_133799 for the cDNA).

25 The gene product of Drosophila Klp67A (Kinesin-like protein at 67A;
GadFly Accession Number CG10923, GenBank Accession Number
NM_079268 for the cDNA, NP_523992 for the protein) is 61%
homologous to human hypothetical protein DKFZp434G2226 (GenBank
Accession Number NP_112494.2 for the protein, NM_031217 for the
30 cDNA). Drosophila Klp67A also shows homology on protein level to mouse
kinesin superfamily protein 18A (GenBank Accession Number NM_139303
for the cDNA).

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The gene product of *Drosophila* CG10133 (GadFly Accession Number, GenBank Accession Numbers AY122113 for the cDNA, AAM52625, and AAF49813 for the protein) is 64% homologous to human unknown protein (protein for MGC:39228; GenBank Accession Number AAH25290.1 for the protein, BC025290 for the cDNA), 61% homologous to human unnamed
5 protein product (GenBank Accession Number BAA91385.1 for the protein, AK000814 for the cDNA), and 59% homologous to human phospholipase A2, group IVB (GenBank Accession Number NP_005081 for the protein, NM_005090 for the cDNA). *Drosophila* CG10133 also shows homology on
10 protein level to mouse protein similar to phospholipase A2, group IVB (GenBank Accession Number XM_130446 for the cDNA).

The gene product of *Drosophila* CG3967 (GadFly Accession Number) is 61% homologous to human hypothetical protein FLJ13158 (GenBank
15 Accession Number XP_042375.2 for the protein, XM_042375 for the cDNA), and 61% homologous to human hypothetical protein FLJ13158 (GenBank Accession Number NP_079185.1 for the protein, NM_024909 for the cDNA). *Drosophila* CG3967 also shows homology on protein level to mouse RIKEN cDNA 3110080J08 and RIKEN cDNA 3110080J08
20 (GenBank Accession Numbers NM_028476 and XM_128622 for the cDNAs, respectively).

The gene product of *Drosophila* CG32048 (GadFly Accession Number CG32048-PA) is 58% homologous to human protein similar to C-terminal
25 PDZ domain ligand of neuronal nitric oxide synthase (GenBank Accession Number XP_034002.1 for the protein, XM_034002 for the cDNA), and 58% homologous to human KIAA0464 protein (GenBank Accession Number BAA32309.2 for the protein, AB007933 for the cDNA). *Drosophila* CG32048 also shows homology on protein level to mouse protein
30 (GenBank Accession Number XM_129577 for the cDNA).

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The gene product of *Drosophila* CG31605 (GadFly Accession Number CG31605-PG) is 45% homologous to human basigin; collagenase stimulatory factor; M6 antigen; extracellular matrix metalloproteinase inducer (GenBank Accession Number NP_001719.1 for the protein, 5 NM_001728 for the cDNA), 46% homologous to human emmprin (GenBank Accession Number BAB88938.1 for the protein, AB072923 for the cDNA), 42% homologous to human stromal cell derived factor receptor 1 isoform b (GenBank Accession Number NP_036560.1 for the protein, NM_012428 for the cDNA), and 42% homologous to human stromal cell 10 derived factor receptor 1 isoform a (GenBank Accession Number NP_059429.1 for the protein, NM_017455 for the cDNA). *Drosophila* CG31605 also shows homology on protein level to mouse basigin (GenBank Accession Number XM_125721 for the cDNA) and to mouse stromal cell derived factor receptor 1 (GenBank Accession Number 15 NM_009145).

The gene product of *Drosophila* Baldspot (GadFly Accession Number CG3971, GenBank Accession Numbers NM_140652 for the cDNA, MP_648909 and AAF49430 for the protein) is 63% homologous to human 20 long-chain fatty-acyl elongase (GenBank Accession Number NP_076995.1 for the protein, NM_024090 for the cDNA), 62% homologous to human protein similar to CIG30 (GenBank Accession Number XP_058360.1 for the protein, XM_058360 for the cDNA), and 66% homologous to human Elongation of very long chain fatty acids protein 3 (Cold inducible 25 glycoprotein of 30 kDa; GenBank Accession Number Q9HB03). *Drosophila* Baldspot also shows homology on protein level to mouse long chain fatty acyl elongase (GenBank Accession Number NM_130450 for the cDNA) and mouse elongation of very long chain fatty acids-like 3 protein (GenBank Accession Number NM_007703 for the cDNA).

30 The gene product of *Drosophila* CG13625 (GadFly Accession Number; GenBank Accession Numbers NM_143015 for the cDNA, NP_651272 for

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the protein) is 50%/39% homologous to human unnamed protein product (GenBank Accession Number BAB71593.1 for the protein, AK057832 for the cDNA) and 42% homologous to human hypothetical protein MGC13125 (GenBank Accession Number NP_116114.1 for the protein, NM_032725 for the cDNA). *Drosophila* CG13625 also shows homology on protein level to mouse hypothetical protein MGC13125 (GenBank Accession Number XM_134776 for the cDNA).

The gene product of *Drosophila fusilli* (*fus*; *GadFly* Accession Number CG8205; GenBank Accession Number NM_079952 for the cDNA, NP_524691 for the protein) is 63%/46% homologous to human protein similar to *fusilli*; enhancer of cactus (GenBank Accession Number XP_007770.7 for the protein, XM_007770 for the cDNA), and 51% homologous to human hypothetical protein FLJ21918 (GenBank Accession Number NP_079215.1 for the protein, NM_024939 for the cDNA). *Drosophila fusilli* also shows homology on protein level to mouse *fusilli*, enhancer of cactus (GenBank Accession Number XM_131317 for the cDNA) and mouse RIKEN cDNA 9530027K23 gene (GenBank Accession Number XM_134507 for the cDNA).

The gene product of *Drosophila schnurri* (*shn*; *GadFly* Accession Number CG7734, GenBank Accession Numbers NM_057375 for the cDNA, NP_476723 for the protein) is 50% homologous to human immunodeficiency virus type 1 enhancer binding protein 1 (GenBank Accession Number NP_002105.1 for the protein, NM_002114 for the cDNA), 54% homologous to human immunodeficiency virus type 1 enhancer binding protein 2 (GenBank Accession Number NP_006725.2 for the protein, NM_006734 for the cDNA), 42% homologous to human protein similar to human immunodeficiency virus type 1 enhancer-binding protein 3 (GenBank Accession Number XP_046509.7 for the protein, XM_046509 for the cDNA), 42% homologous to human KIAA1555 protein (GenBank Accession Number BAB13381.1 for the protein, AB046775 for

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the cDNA), and 43% homologous to human protein similar to dJ492J12.1 (novel protein similar to zinc finger protein human immunodeficiency virus type I enhancer-binding protein 1; GenBank Accession Number XP_066058.1 for the protein, XM_066058 for the cDNA). *Drosophila* schnurri also shows homology on protein level to several mouse proteins (GenBank Accession Numbers NM_007772, NM_010437, NM_010657, and XM141574 for the cDNA).

cGMP-dependent protein kinase, type I is also referred to in patent application WO0255664. Solute carrier family 7, member 1 is also referred to in patent application WO9210506, in patent US5834589, and in patent application WO9325682. Prostate derived STE20-like kinase PSK is also referred to in patent application WO0058473 and KIAA1361 protein is also referred to in patent application WO0222660. Stromal cell derived factor receptor 1 is also referred to in patent application WO0029583. ELOVL3 is also referred to in patent application WO0134643, in patent application WO0230974, and in patent application WO0244320. ELOVL family member 6 is referred to in patent application WO0118016, in patent application WO0208401, in patent application WO0012720, and in patent application WO0065054.

Example 4: Genetic ucp pathway screen

Expression of *Drosophila* uncoupling protein dUCPy in a non-vital organ like the eye (Gal4 under control of the eye-specific promoter of the *eyeless* gene) results in flies with visibly damaged eyes. This easily visible eye phenotype is the basis of a genetic screen for gene products that can modify UCP activity.

Parts of the genomes of the strain with Gal4 expression in the eye and the strain carrying the pUAST-dUCPy construct were combined on one

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chromosome using genomic recombination. The resulting fly strain has eyes that are permanently damaged by dUCPy expression. Flies of this strain were crossed with flies of a large collection of mutagenized fly strains. In this mutant collection a special expression system (EP-element, Ref.: Rorth P., (1996) Proc Natl Acad Sci U S A, 93: 12418-12422) is integrated randomly in different genomic loci. The yeast transcription factor Gal4 can bind to the EP-element and activate the transcription of endogenous genes close the integration site of the EP-element. The activation of the genes therefore occurs in the same cells (eye) that overexpress dUCPy. Since the mutant collection contains several thousand strains with different integration sites of the EP-element it is possible to test a large number of genes whether their expression interacts with dUCPy activity. In case a gene acts as an enhancer of UCP activity the eye defect will be worsened; a suppressor will ameliorate the defect.

Using this screen a gene with enhancing activity was discovered that was found to be the CG32048 gene in *Drosophila*, and a gene with suppressing activity was discovered that was found to be the *schnurri* gene (GadFly Accession Number CG7734) in *Drosophila*.

Example 5: Genetic adp pathway screen

Adipose (adp) is a protein that has been described as regulating, causing or contributing to obesity in an animal or human (see WO 01/96371). Transgenic flies containing a wild type copy of the adipose cDNA under the control of the Gal4/UAS system were generated (Brand A.H. and Perrimon N., (1993) Development 118: 401-415; for adipose cDNA, see WO 01/96371).

Chromosomal recombination of these transgenic flies with an *eyeless*-Gal4 driver line has been used to generate a stable recombinant fly line (herein

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referred to as eye-adp-Gal4) over-expressing adipose in the developing *Drosophila* eye. Animals receiving transgenic adipose activity under these conditions developed into adult flies with a visible change of eye phenotype. Virgins of the recombinant driver line were crossed with males
5 of the mutant EP-line collection in single crosses and kept for preferably 12 to 15 days at 29°C. The offspring was checked for modifications of the eye phenotype (enhancement or suppression). Mutations changing the eye phenotype affect genes that modify adipose activity. The inventors have found that the fly lines HD-EP(3)36956, HD-EP(3)36681, and
10 HD-EP(3)36364 are enhancers of the eye-adp-Gal4 induced eye phenotype.

Chromosomal recombination of the above mentioned transgenic flies, containing a wild type copy of the adipose cDNA under the control of the Gal4/UAS system, with an FB-Gal4 driver line has been used to generate a
15 stable recombinant fly line (herein referred to as FB-adp-Gal4) with ectopic expression of adipose mainly in the fatbody of these flies. At the permissive temperature (preferably 18°C) animals receiving transgenic adipose activity under these conditions develop into adult flies. The restrictive temperature (preferably 25°C) leads to lethality of animals
20 receiving transgenic adipose activity under these conditions (lethality phenotype). However, a small number of animals will survive the restrictive temperature and hatch (these are referred to as 'escapers'). Virgins of the recombinant driver line (FB-adp-Gal4) were collected at the permissive temperature, crossed with males of the mutant EP-line collection in single
25 crosses and kept for preferably 15 days at 25°C. In this mutant EP-line collection a special expression system (EP-element, Ref.: Rorth P., (1996), supra) is integrated randomly in different genomic loci. The offspring coexpressing adipose and the endogenous genes close the integration site of the EP-element was checked for modifications of the lethality
30 phenotype. Enhancement of the lethality phenotype was detected by a decreased number of escapers and suppression of the lethality phenotype was detected by increased viability of flies at the restrictive temperature.

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Mutations changing the lethality phenotype affect genes that modify adipose activity. The inventors have found that the fly line HD-EP(3)31600 is an enhancer of the FB-adp-Gal4 induced lethality phenotype, and that the fly lines HD-EP(3)31171, HD-EP(3)30418, and HD-EP(2)22018 are suppressors of the FB-adp-Gal4 induced lethality phenotype.

These results are strongly suggesting an interaction of the CG3967 gene, the CG32048 gene and the schnurri gene with adp since the integrations of HD-EP(3)36956, HD-EP(3)36681 and HD-EP(3)31600 were found to be located at the CG3967 locus, the integrations of HD-EP(3)36364, HD-EP(3)31171, and HD-EP(3)30418 were found to be located at the CG32048 locus, and the integration of HD-EP(2)22018 was found to be located at the schnurri locus. This is suggesting a function of CG3967, CG32048, schnurri, and homologous proteins in the regulation of the energy homeostasis.

Example 6: Expression of the polypeptides in mammalian (mouse) tissues

To analyse the expression of the polypeptides disclosed in this invention in mammalian tissues, several mouse strains (preferably mice strains C57Bl/6J, C57Bl/6 ob/ob and C57Bl/KS db/db which are standard model systems in obesity and diabetes research) were purchased from Harlan Winkelmann (33178 Borcheln, Germany) and maintained under constant temperature (preferably 22°C), 40 per cent humidity and a light / dark cycle of preferably 14 / 10 hours. The mice were fed a standard chow (for example, from ssniff Spezialitäten GmbH, order number ssniff M-Z V1126-000). For the fasting experiment ("fasted wild type mice"), wild type mice were starved for 48 h without food, but only water supplied ad libitum (see, for example, Schnetzler B. et al., 1993, J Clin Invest 92: 272-280, Mizuno T.M. et al., 1996, Proc Natl Acad Sci U S A 93: 3434-3438). In a further experiment wild-type (wt) mice were fed a control

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diet (preferably Altromin C1057 mod control, 4.5% crude fat) or high fat diet (preferably Altromin C1057mod. high fat, 23.5% crude fat). Animals were sacrificed at an age of 6 to 8 weeks. The animal tissues were isolated according to standard procedures known to those skilled in the art, snap frozen in liquid nitrogen and stored at -80°C until needed.

For analyzing the role of the proteins disclosed in this invention in the in vitro differentiation of mammalian cell culture cells for the conversion of pre-adipocytes to adipocytes, mammalian fibroblast (3T3-L1) cells (e.g., Green H. and Kehinde O., 1974, Cell 1: 113-116) were obtained from the American Tissue Culture Collection (ATCC, Hanassas, VA, USA; ATCC- CL 173). 3T3-L1 cells were maintained as fibroblasts and differentiated into adipocytes as described in the prior art (e.g., Qiu Z. et al., 2001, J. Biol. Chem. 276: 11988-11995; Slieker L.J. et al., 1998, BBRC 251: 225-229). In brief, cells were plated in DMEM/10% FCS (Invitrogen, Karlsruhe, Germany) at 50,000 cells/well in duplicates in 6-well plastic dishes and cultured in a humidified atmosphere of 5% CO₂ at 37°C. At confluence (defined as day 0: d0) cells were transferred to serum-free (SF) medium, containing DMEM/HamF12 (3:1; Invitrogen), fetuin (300 µg/ml; Sigma, Munich, Germany), transferrin (2 µg/ml; Sigma), pantothenate (17 µM; Sigma), biotin (1 µM; Sigma), and EGF (0.8 nM; Hoffmann-La Roche, Basel, Switzerland). Differentiation was induced by adding dexamethasone (DEX; 1 µM; Sigma), 3-methyl-isobutyl-1-methylxanthine (MIX; 0.5 mM; Sigma), and bovine insulin (5 µg/ml; Invitrogen). Four days after confluence (d4), cells were kept in SF medium, containing bovine insulin (5 µg/ml) until differentiation was completed. At various time points of the differentiation procedure, beginning with day 0 (day of confluence) and day 2 (hormone addition; for example, dexamethasone and 3-isobutyl-1-methylxanthine), up to 10 days of differentiation, suitable aliquots of cells were taken every two days.

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RNA was isolated from tissues and cells using Trizol Reagent (for example, from Invitrogen, Karlsruhe, Germany) and further purified with the RNeasy Kit (for example, from Qiagen, Germany) in combination with an DNase-treatment according to the instructions of the manufacturers and as
5 known to those skilled in the art. Total RNA was reverse transcribed (preferably using Superscript II RNaseH- Reverse Transcriptase, from Invitrogen, Karlsruhe, Germany) and subjected to Taqman analysis preferably using the Taqman 2xPCR Master Mix (from Applied Biosystems, Weiterstadt, Germany; the Mix contains according to the Manufacturer for
10 example AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference Rox and optimized buffer components) on a GeneAmp 5700 Sequence Detection System (from Applied Biosystems, Weiterstadt, Germany).

15 Taqman analysis was performed preferably using the following primer/probe pairs:

For the amplification of mouse protein kinase, cGMP-dependent, type I (Prkg1) sequence (GenBank Accession Number NM_011160): Mouse Prkg1
20 forward primer (SEQ ID NO: 1): 5'- GCC TAT CTG CAT TCC AAA GGA A-3'; mouse Prkg1 reverse primer (SEQ ID NO: 2): 5'- TGG CAT AGC CTC GAT GAT CTA G-3'; mouse Prkg1 Taqman probe (SEQ ID NO: 3): (5/6-FAM)-CAT TTA CAG GGA CCT CAA GCC GGA GAA-(5/6-TAMRA).

25 For the amplification of mouse protein kinase, cGMP-dependent, type II (Prkg2) sequence (GenBank Accession Number NM_008926): Mouse Prkg2 forward primer (SEQ ID NO: 4): 5'- CAG GCC TCT CCC GAC AAA -3'; mouse Prkg2 reverse primer (SEQ ID NO: 5): 5'- GCT GTG GAG CGA GAC CAA G-3'; mouse Prkg2 Taqman probe (SEQ ID NO: 6): (5/6-FAM)- TGC
30 CTC TGG ATG TTC ACC GCA AGA C- (5/6-TAMRA).

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For the amplification of mouse solute carrier family 7 (cationic amino acid transporter, γ^+ system), member 1 (Slc7a1) sequence (GenBank Accession Number NM_007513): Mouse Slc7a1 forward primer (SEQ ID NO: 7): 5'- GTG GCT GCC TGT GTT TTG GT-3'; mouse Slc7a1 reverse
5 primer (SEQ ID NO: 8): 5'- TCG GTG GTT CTG GCC ATC-3'; mouse Slc7a1 Taqman probe (SEQ ID NO: 9): (5/6-FAM)-ACG GTA CCA GCC AGA ACA ACC TAA TCT GGT A- (5/6-TAMRA).

For the amplification of mouse solute carrier family 7 (cationic amino acid
10 transporter, γ^+ system), member 2 (Slc7a2) sequence (GenBank Accession Number NM_007514): Mouse Slc7a2 forward primer (SEQ ID NO: 10): 5'- CTG GCT CTT CTC GCC CTG T -3'; mouse Slc7a2 reverse primer (SEQ ID NO: 11): 5'- GAT TCT GTG GCT GCC TCC A-3'; mouse Slc7a2 Taqman probe (SEQ ID NO: 12): (5/6-FAM)- CTC TGC GCT GCC
15 GTC ATT CTG ACC -(5/6-TAMRA).

For the amplification of mouse solute carrier family 7 (cationic amino acid transporter, γ^+ system), member 3 (Slc7a3) sequence (GenBank Accession Number NM_007515): Mouse Slc7a3 forward primer (SEQ ID
20 NO: 13): 5'- TGC CTC GGG TGA TGT ATT CAA-3'; mouse Slc7a3 reverse primer (SEQ ID NO: 14): 5'- ATG TGT GAC ACT GTG AAC CTT AGC A-3'; mouse Slc7a3 Taqman probe (SEQ ID NO: 15): (5/6-FAM)-CAG AAG ATG GCC TCC TGT TCC GTG TC-(5/6-TAMRA).

25 For the amplification of mouse RIKEN cDNA 1110033K02 gene (1110033K02Rik) sequence (GenBank Accession Number XM_133799): Mouse 1110033K02Rik forward primer (SEQ ID NO: 16): 5'- GAG CAA CGG GTG GAA GAA GA -3'; mouse 1110033K02Rik reverse primer (SEQ ID NO: 17): 5'- AAA CTC CGG ATA CGT TCC GA-3'; mouse
30 1110033K02Rik Taqman probe (SEQ ID NO: 18): (5/6-FAM)-CTG CTG GCC CTA CAG ACA GGC CG-(5/6-TAMRA).

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For the amplification of mouse similar to thousand and one amino acid protein kinase (LOC192767) sequence (GenBank Accession Number XM_109632): Mouse LOC192767 forward primer (SEQ ID NO: 19): 5'-GAA CAT GCA ATG CTA TTG CGA C-3'; mouse LOC192767 reverse
5 primer (SEQ ID NO: 20): 5'- CTG ATC AAC TCA CAG CGC ATC-3'; mouse LOC192767 Taqman probe (SEQ ID NO: 21): (5/6-FAM)-ACT GGA GTT TCG CCA TCT CAA CAC TAT TCA GA-(5/6-TAMRA).

For the amplification of mouse kinesin family member 18A (Kif18a)
10 sequence (GenBank Accession Number NM_139303): Mouse Kif18a forward primer (SEQ ID NO: 22): 5'- AAT GGC ACA CAT GAC AGC TCT AG-3'; mouse Kif18a reverse primer (SEQ ID NO: 23): 5'- AAG CAA AGC ATT CAA TAC TGC CT-3'; mouse Kif18a Taqman probe (SEQ ID NO: 24): (5/6-FAM)-TCT CCA GGA ACA GCA GCA CAA GCA AAC T-(5/6-TAMRA).

For the amplification of mouse similar to phospholipase A2, group IVB (cytosolic) (LOC211429) sequence (GenBank Accession Number XM_130446): Mouse LOC211429 forward primer (SEQ ID NO: 25): 5'-
20 TGC CAG CTC TGT GGT TCC A-3'; mouse LOC211429 reverse primer (SEQ ID NO: 26): 5'- ATG TCA TAC CAG AAA TTC ACA GCA A-3'; mouse LOC211429 Taqman probe (SEQ ID NO: 27): (5/6-FAM)-CAT GTC CAG CAG TCC CAC GGC TG-(5/6-TAMRA).

For the amplification of mouse ELOVL family member 6, elongation of long chain fatty acids (yeast) (Elovl6) sequence (GenBank Accession Number NM_130450): Mouse Elovl6 forward primer (SEQ ID NO: 28): 5'- GAA CTA TGG CGT GCA TGC C-3'; mouse Elovl6 reverse primer (SEQ ID NO: 29): 5'- CGG GAG ACT CGG AAA CCC-3'; mouse Elovl6 Taqman probe
30 (SEQ ID NO: 30): (5/6-FAM)-ACT ACG CCT TGC GGG CTG CG-(5/6-TAMRA).

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For the amplification of mouse elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3 (Elovl3) sequence (GenBank Accession Number NM_007703): Mouse Elovl3 forward primer (SEQ ID NO: 31): 5'- CAG GTG GCG TCG CAG C-3'; mouse Elovl3 reverse primer (SEQ ID NO: 32): 5'- GAG TAT GAA GCT TGG TGT GAC CAG-3'; mouse Elovl3 Taqman probe (SEQ ID NO: 33): (5/6-FAM)-CCA GCA GAG AAC GAT CCC TGT CAG C-(5/6-TAMRA).

In the figures the relative RNA-expression is shown on the Y-axis. In Figures 4 A-C, 4E-G, 9A-C, 9E-G, 9I-K, 13A-C, 13E-G, 16A-C, 19A-B, 29A-C, and 29E-G, the tissues tested are given on the X-axis. "WAT" refers to white adipose tissue, "BAT" refers to brown adipose tissue. In Figure 4D, 4H, 9D, 9H, 9L, 13D, 13H, 16D, 19C, 29D, and 29H, the X-axis represents the time axis. "d0" refers to day 0 (start of the experiment), "d2" - "d12" refers to day 2 - day 12 of adipocyte differentiation.

The function of the proteins of the invention in metabolism was further validated by analyzing the expression of the transcripts in different tissues and by analyzing the role in adipocyte differentiation.

In one embodiment of this invention, mouse models of insulin resistance and/or diabetes were used, such as mice carrying gene knockouts in the leptin pathway (for example, ob/ob (leptin) or db/db (leptin receptor/ligand) mice) to study the expression of the proteins of the invention. Such mice develop typical symptoms of diabetes, show hepatic lipid accumulation and frequently have increased plasma lipid levels (see Bruning J.C. et al, (1998) Mol. Cell. 2: 559-569).

In a further embodiment of the invention, expression of the mRNAs encoding the proteins of the invention was also examined in susceptible

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wild type mice (for example, C57Bl/6) that show symptoms of diabetes, lipid accumulation, and high plasma lipid levels, if fed a high fat diet.

Expression profiling studies confirm the particular relevance of Prkg1 as
5 regulator of energy metabolism in mammals. Taqman analysis revealed that
Prkg1 is expressed in several mammalian tissues, showing highest level of
expression in muscle and higher levels in further tissues, e.g. lung,
hypothalamus, brain, heart and testis. Furthermore Prkg1 is expressed on
lower but still robust levels in WAT, BAT and kidney of wild type mice as
10 depicted in Figure 4A. We found, for example, that the expression of Prkg1
is up regulated in WAT, BAT and hypothalamus of genetically induced
obese mice (ob/ob) compared to wild type mice. Furthermore Prkg1 is up
regulated in BAT of fasted mice compared to wild type mice (see Figure
4B). In wild type mice fed a high fat diet, the expression of Prkg1 is up
15 regulated in BAT as depicted in Figure 4C. We show in this invention (see
Figure 4D) that the Prkg1 mRNA is expressed and down regulated during
the differentiation into mature adipocytes. Therefore, the Prkg1 protein
might play an essential role in adipogenesis.

20 The expression of Prkg1 in metabolic active tissues of wild type mice, as
well as the up regulation of Prkg1 in different animal models used to study
metabolic disorders, suggests that this gene plays a central role in energy
homeostasis. This hypothesis is supported by the down regulation during
the differentiation from preadipocytes to mature adipocytes,

25 Expression profiling studies confirm the particular relevance of Prkg2 as
regulator of energy metabolism in mammals. Taqman analysis revealed
highest level of Prkg2 expression in the small intestine of wild type mice.
Furthermore Prkg2 is expressed on high levels in hypothalamus, brain, lung
and colon of wild type mice as depicted in Figure 4E. We found, for
30 example, that the expression of Prkg2 is up regulated in the hypothalamus
of genetically induced obese mice (ob/ob) compared to wild type mice (see

Figure 4F). In wild type mice fed a high fat diet, the expression of Prkg2 is up regulated in WAT, BAT and lung and down regulated in small intestine as depicted in Figure 4G. We show in this invention (see Figure 4H) that the Prkg2 mRNA is expressed and down regulated during the differentiation into mature adipocytes. Therefore, the Prkg2 protein might play an essential role in adipogenesis.

The regulated expression of Prkg2 in different animal models used to study metabolic disorders, together with the down regulation during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

Expression profiling studies confirm the particular relevance of Slc7a1 as regulator of energy metabolism in mammals. Taqman analysis revealed that Slc7a1 is expressed in several mammalian tissues, showing highest level of expression in WAT and higher levels in further tissues, e.g. hypothalamus, brain, heart, lung, small intestine, spleen, colon and testis. Furthermore Slc7a1 is expressed on lower but still robust levels in BAT, muscle, pancreas and kidney of wild type mice as depicted in Figure 9A. We found, for example, that the expression of Slc7a1 is down regulated in WAT and up regulated in BAT and liver of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore Slc7a1 is up regulated in liver and down regulated in bone marrow of fasted mice compared to wild type mice (see Figure 9B). In wild type mice fed a high fat diet, the expression of Slc7a1 is up regulated in BAT and muscle and down regulated in the WAT as depicted in Figure 9C. We show in this invention (see Figure 9D) that the Slc7a1 mRNA is expressed and regulated during the differentiation into mature adipocytes. Therefore, the Slc7a1 protein might play an essential role in adipogenesis.

The expression of Slc7a1 is strongly regulated in metabolic active tissues (e.g. WAT, BAT, muscle and liver) of different animal models used to study

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metabolic disorders. The down regulated expression of Slc7a1 in the WAT of both, genetically and diet induced obese mice, along with the up regulation of Slc7a1 in the BAT of obese mice, suggests that this gene plays a central role in energy homeostasis.

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Expression profiling studies confirm the particular relevance of Slc7a2 as regulator of energy metabolism in mammals. Taqman analysis revealed highest level of Slc7a2 expression in liver of wild type mice. Furthermore Slc7a2 is expressed on lower but still robust levels in WAT, muscle, lung, hypothalamus, brain, spleen and kidney of wild type mice as depicted in Figure 9E. We found, for example, that the expression of Slc7a2 is up regulated in the spleen of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore Slc7a2 is up regulated in muscle and liver of fasted mice compared to wild type mice (see Figure 9F). In wild type mice fed a high fat diet, the expression of Slc7a2 is down regulated in spleen and kidney as depicted in Figure 9G. We show in this invention (see Figure 9H) that the Slc7a2 mRNA is expressed and regulated during the differentiation into mature adipocytes. Therefore, the Slc7a2 protein might play an essential role in adipogenesis.

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The regulated expression of Slc7a2 in metabolic active tissues of different animal models used to study metabolic disorders, together with the expression of Slc7a2 during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

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Expression profiling studies confirm the particular relevance of Slc7a3 as regulator of energy metabolism in mammals. Taqman analysis revealed highest level of Slc7a3 expression in hypothalamus and brain of wild type mice. Furthermore Slc7a3 is expressed on lower but still robust levels in WAT, BAT, pancreas and lung of wild type mice as depicted in Figure 9I. We found, for example, that the expression of Slc7a3 is down regulated in

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the WAT of genetically induced obese mice (ob/ob) compared to wild type mice (see Figure 9J). We show in this invention (see Figure 9K) that the Slc7a3 mRNA is expressed and regulated during the differentiation into mature adipocytes. Therefore, the Slc7a3 protein might play an essential
5 role in adipogenesis.

The down regulation of Slc7a3 expression in a metabolic active tissues of genetically obese mice, together with the expression of Slc7a3 during the differentiation from preadipocytes to mature adipocytes, suggests that this
10 gene plays a central role in energy homeostasis.

Expression profiling studies confirm the particular relevance of 1110033K02Rik as regulator of energy metabolism in mammals. Taqman analysis revealed that 1110033K02Rik is expressed in several mammalian
15 tissues, showing highest level of expression in hypothalamus, brain, and testis and higher levels in further tissues, e.g., lung, WAT, spleen, and kidney. Furthermore 1110033K02Rik is expressed on lower but still robust levels in heart, BAT, muscle, and liver of wild type mice as depicted in Figure 13A. We found, for example, that the expression of
20 1110033K02Rik is down regulated in WAT of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore 1110033K02Rik is down regulated in WAT of fasted mice compared to wild type mice (see Figure 13B). In wild type mice fed a high fat diet, the expression of 1110033K02Rik is up regulated in muscle as depicted in Figure 13C. We
25 show in this invention (see Figure 13D) that the 1110033K02Rik mRNA is expressed and down regulated during the differentiation into mature adipocytes. Therefore, the 1110033K02Rik protein might play an essential role in adipogenesis.

30 The regulated expression of 1110033K02Rik in metabolic active tissues (e.g. WAT and muscle) of different animal models used to study metabolic disorders, together with the down regulation of 1110033K02Rik

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expression during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

5 Expression profiling studies confirm the particular relevance of LOC192767 as regulator of energy metabolism in mammals. Taqman analysis revealed that LOC192767 is expressed in several mammalian tissues, showing highest level of expression in hypothalamus, brain, and testis and higher levels in further tissues, e.g. BAT, WAT, lung, spleen, muscle, and kidney.
10 Furthermore LOC192767 is expressed on lower but still robust levels in liver, colon, small intestine, pancreas, and bone marrow of wild type mice as depicted in Figure 13E. We found, for example, that the expression of LOC192767 is up regulated in the spleen of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore LOC192767 is down
15 regulated in bone marrow of fasted mice compared to wild type mice (see Figure 13F). In wild type mice fed a high fat diet, the expression of LOC192767 is up regulated in WAT as depicted in Figure 13G. We show in this invention (see and Figure 13H) that the LOC192767 mRNA is expressed and down regulated during the differentiation into mature
20 adipocytes. Therefore, the LOC192767 protein might play an essential role in adipogenesis.

The up regulation of LOC192767 expression in a metabolic active tissue (WAT) of mice fed a high fat diet, together with the down regulation of
25 LOC192767 expression during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

Expression profiling studies confirm the particular relevance of Kif18a as
30 regulator of energy metabolism in mammals. Taqman analysis revealed highest level of Kif18a expressed in testis. Furthermore Kif18a is expressed on lower but still robust levels in several other tissues, e.g. in spleen, bone

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marrow, colon, small intestine, WAT, BAT, and muscle of wild type mice as depicted in Figure 16A. We found, for example, that the expression of Kif18a is up regulated in WAT and BAT of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore Kif18a is up regulated in pancreas of fasted mice compared to wild type mice (see Figure 16B). In wild type mice fed a high fat diet, the expression of Kif18a is up regulated in BAT and muscle as depicted in Figure 16C. We show in this invention (see Figure 16D) that the Kif18a mRNA is expressed and regulated during the differentiation into mature adipocytes. Therefore, the Kif18a protein might play an essential role in adipogenesis.

The regulated expression of Kif18a in metabolic active tissues of different animal models used to study metabolic disorders, together with the regulated expression of Kif18a during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

Expression profiling studies confirm the particular relevance of LOC211429 as regulator of energy metabolism in mammals. Taqman analysis revealed that LOC211429 is expressed in several mammalian tissues, showing highest level of expression in hypothalamus and testis, and higher levels in further tissues, e.g. BAT, heart, lung, spleen, brain, and kidney. Furthermore LOC211429 is expressed on lower but still robust levels in WAT, muscle, liver, colon, small intestine, and bone marrow of wild type mice as depicted in Figure 19A. We found, for example, that the expression of LOC211429 is down regulated in the bone marrow of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore LOC211429 is down regulated in bone marrow of fasted mice compared to wild type mice (see Figure 19B). We show in this invention (see Figure 19C) that the LOC211429 mRNA is expressed and down

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regulated during the differentiation into mature adipocytes. Therefore, the LOC211429 protein might play an essential role in adipogenesis.

5 The expression of LOC211429 in metabolic active tissues of wild type mice, together with the down regulation of LOC211429 expression during the differentiation from preadipocytes to mature adipocytes, suggests that this gene plays a central role in energy homeostasis.

10 Expression profiling studies confirm the particular relevance of Elovl6 as regulator of energy metabolism in mammals. Taqman analysis revealed that Elovl6 is expressed in several mammalian tissues, showing highest level of expression in BAT and higher levels in further tissues, e.g. small intestine, hypothalamus, brain WAT, liver, and kidney. Furthermore Elovl6 is expressed on lower but still robust levels in muscle of wild type mice as depicted in Figure 29A. We found, for example, that the expression of
15 Elovl6 is strongly up regulated in the liver and down regulated in BAT of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore Elovl6 is down regulated in BAT, muscle, and liver of fasted mice compared to wild type mice (see Figure 29B). In wild type mice fed a
20 high fat diet, the expression of Elovl6 is down regulated in WAT, lung, and heart as depicted in Figure 29C. We show in this invention (see Figure 29D) that the Elovl6 mRNA is expressed and down regulated at a very early time point during the differentiation into mature adipocytes. Therefore, the Elovl6 protein might play an essential role in adipogenesis.

25 The strong up regulation of Elovl6 expression in the liver of genetically obese mice, together with the down regulation in the same tissue of fasted mice, suggests that this gene plays a central role in energy homeostasis. This hypothesis is supported by the regulated expression of Elovl6 in
30 further metabolic active tissues (WAT, BAT and muscle) of different animal models used to study metabolic disorders and by the regulated expression

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of Elovl6 during the differentiation from preadipocytes to mature adipocytes.

5 Expression profiling studies confirm the particular relevance of Elovl3 as regulator of energy metabolism in mammals. Taqman analysis revealed that Elovl3 is expressed in specific mammalian tissues, showing highest level of expression in the liver. Furthermore Elovl3 is expressed on lower but still robust levels in BAT and muscle of wild type mice as depicted in Figure 29E. We found, for example, that the expression of Elovl3 is down
10 regulated in WAT, BAT, and liver of genetically induced obese mice (ob/ob) compared to wild type mice. Furthermore Elovl3 is down regulated in liver of fasted mice compared to wild type mice (see Figure 29F). In wild type mice fed a high fat diet, the expression of Elovl3 is up regulated in WAT and small intestine as depicted in Figure 29G. We show in this invention
15 (see Figure 29H) that the Elovl3 mRNA is strongly up regulated during the differentiation into mature adipocytes. Therefore, the Elovl3 protein might play an essential role in adipogenesis.

The regulated expression of Elovl3 in metabolic active tissues (WAT, BAT
20 and liver) of different animal models used to study metabolic disorders, suggests that this gene plays a central role in energy homeostasis. This hypothesis is supported by the strong up regulation of Elovl3 expression during the differentiation from preadipocytes to mature adipocytes.

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Example 7. Analysis of the differential expression of transcripts of the proteins of the invention in human tissues

30 RNA preparation from human primary adipose tissues was done as described in Example 6. The hybridization and scanning was performed as described in the manufactures manual (see Affymetrix Technical Manual, 2002, obtained from Affmetrix, Santa Clara, USA).

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In Figures 5, 26, 30, and 37, the X-axis represents the time axis, shown are day 0 and day 12 of adipocyte differentiation. The Y-axis represents the fluorescent intensity. The expression analysis (using Affymetrix GeneChips) of the cGMP-dependent protein kinase, type II (PRKG2), stromal cell derived factor receptor 1 (SDFR1), basigin (BSG), ELOVL family member 6, elongation of long chain fatty acids (ELOVL6), and immunodeficiency virus type I enhancer binding protein 1 (HIVEP1) genes using primary human abdominal adipocyte differentiation clearly shows differential expression of human PRKG2, SDFR1, BSG, ELOVL6, and HIVEP1 genes in adipocytes. Several independent experiments were done. The experiments further show that the PRKG2, SDFR1, and HIVEP1 transcripts (see Figures 5, 26A, and 37) are most abundant at day 0 compared to day 12 during differentiation, and that the BSG and ELOVL6 transcripts (see Figures 26B and 30) are most abundant at day 12 compared to day 0 during differentiation.

Thus, the PRKG2, SDFR1, and HIVEP1 proteins have to be significantly decreased and the BSG and ELOVL6 proteins have to be significantly increased in order for the preadipocytes to differentiate into mature adipocyte. Therefore, the PRKG2, SDFR1, and HIVEP1 proteins in preadipocytes have the potential to inhibit, and the BSG and ELOVL6 proteins in preadipocytes have the potential to enhance adipose differentiation. Therefore, PRKG2, SDFR1, BSG, ELOVL6, and HIVEP1 proteins might play an essential role in the regulation of human metabolism, in particular in the regulation of adipogenesis and thus it might play an essential role in obesity, diabetes, and/or metabolic syndrome.

For the purpose of the present invention, it will be understood by the person having average skill in the art that any combination of any feature mentioned throughout the specification is explicitly disclosed herewith.

Claims

1. A pharmaceutical composition comprising a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous protein and/or a functional fragment thereof, a nucleic acid molecule encoding a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous protein and/or a functional fragment thereof and/or a modulator/effector of said nucleic acid molecule and/or said protein together with pharmaceutically acceptable carriers, diluents and/or additives.
2. The composition of claim 1, wherein the nucleic acid molecule is a vertebrate or insect foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri nucleic acid, particularly encoding a human protein as described in Table 1, and/or a nucleic molecule which is complementary thereto or a functional fragment thereof or a variant thereof.
3. The composition of claim 1 or 2, wherein said nucleic acid molecule is selected from the group consisting of
 - (a) a nucleic acid molecule encoding a polypeptide as shown in Table 1, or an isoform, fragment or variant of said polypeptide;
 - (b) a nucleic acid molecule which comprises or is the nucleic acid molecule as shown in Table 1;
 - (c) a nucleic acid molecule being degenerate as a result of the genetic code to the nucleic acid sequences as defined in (a) or (b);

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- (d) a nucleic acid molecule that hybridizes at 50°C in a solution containing 1 x SSC and 0.1% SDS to a nucleic acid molecule as defined in claim 2 or as defined in (a) to (c) and/or a nucleic acid molecule which is complementary thereto;
- 5 (e) a nucleic acid molecule that encodes a polypeptide which is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably at least 98% and up to 99,6% identical to a human protein as described in Table 1 or as defined in claim 2 or to a polypeptide as defined in (a);
- 10 (f) a nucleic acid molecule that differs from the nucleic acid molecule of (a) to (e) by mutation and wherein said mutation causes an alteration, deletion, duplication or premature stop in the encoded polypeptide.
- 15 4. The composition of any one of claims 1-3, wherein the nucleic acid molecule is a DNA molecule, particularly a cDNA or a genomic DNA.
5. The composition of any one of claims 1-4, wherein said nucleic acid encodes a polypeptide contributing to regulating the energy homeostasis and/or the metabolism of triglycerides.
- 20 6. The composition of any one of claims 1-5, wherein said nucleic acid molecule is a recombinant nucleic acid molecule.
- 25 7. The composition of any one of claims 1-6, wherein the nucleic acid molecule is a vector, particularly an expression vector.
8. The composition of any one of claims 1-5, wherein the polypeptide is a recombinant polypeptide.
- 30 9. The composition of claim 8, wherein said recombinant polypeptide is a fusion polypeptide.

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10. The composition of any one of claims 1-7, wherein said nucleic acid molecule is selected from hybridization probes, primers and anti-sense oligonucleotides.
- 5 11. The composition of any one of claims 1-10 which is a diagnostic composition.
12. The composition of any one of claims 1-10 which is a therapeutic composition.
- 10 13. The composition of any one of claims 1-12 for the manufacture of an agent for detecting and/or verifying, for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis, in cells, cell masses, organs and/or subjects.
- 15 14. Use of a nucleic acid molecule as described in Table 1, particular of a nucleic acid molecule according to claim 3 (a), (b), or (c), or a polypeptide encoded thereby or a functional fragment or a variant of said nucleic acid molecule or said polypeptide and/or a modulator/effector of said nucleic acid molecule or polypeptide for the manufacture of a medicament for the treatment of obesity, diabetes, and/or metabolic syndrome for controlling the function of a gene and/or a gene product which is influenced and/or modified by a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly by a polypeptide according to claim 3.
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15. Use of the nucleic acid molecule as described in Table 1, particularly of a nucleic acid molecule according to claim 3 (a), (b), or (c), or a polypeptide encoded thereby or a fragment or a variant of said nucleic acid molecule or said polypeptide or a modulator/effector of said nucleic acid molecule or said polypeptide for identifying substances capable of interacting with a polypeptide, particularly with a polypeptide according to claim 3.
16. A non-human transgenic animal exhibiting a modified expression of a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly of a polypeptide according to claim 3.
17. The animal of claim 16, wherein the expression of the foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly of a polypeptide according to claim 3, is increased and/or reduced.
18. A recombinant host cell exhibiting a modified expression of a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly of a polypeptide according to claim 3.
19. The cell of claim 18 which is a human cell.
20. A method of identifying a (poly)peptide involved in the regulation of energy homeostasis and/or metabolism of triglycerides in a mammal comprising the steps of

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- 5 (a) contacting a collection of (poly)peptides with a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly with a polypeptide according to claim 3, or a functional fragment thereof under conditions that allow binding of said (poly)peptides;
- (b) removing (poly)peptides which do not bind and
- 10 (c) identifying (poly)peptides that bind to said foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide.

21. A method of screening for an agent which modulates/effects the interaction of a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly of a polypeptide according to claim 3, with a binding target, comprising the steps of
- 15 (a) incubating a mixture comprising
- (aa) a foraging, CG5535, CG14217, Klp67A, CG10133, G3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly a polypeptide according to claim 3, or a functional fragment thereof;
- 20 (ab) a binding target/agent of said polypeptide or functional fragment thereof; and
- 25 (ac) a candidate agent under conditions whereby said polypeptide or functional fragment thereof specifically binds to said binding target/agent at a reference affinity;
- 30 (b) detecting the binding affinity of said polypeptide or functional fragment thereof to said binding target to determine an affinity for the agent; and

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- (c) determining a difference between affinity for the agent and the reference affinity.

22. A method for screening for an agent, which modulates/effects the activity of a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous polypeptide, particularly of a polypeptide according to claim 3, comprising the steps of

(a) incubating a mixture comprising

(aa) said polypeptide or a functional fragment thereof;

(ab) a candidate agent

under conditions whereby said polypeptide or functional fragment thereof has a reference activity;

(b) detecting the activity of said polypeptide or functional fragment thereof to determine an activity in presence of the agent; and

(c) determining a difference between the activity in the presence of the agent and the reference activity.

23. A method of producing a composition comprising the (poly)peptide identified by the method of claim 20 or the agent identified by the method of claim 21 or 22 with a pharmaceutically acceptable carrier, diluent and/or additive.

24. The method of claim 23 wherein said composition is a pharmaceutical composition for preventing, alleviating or treating of metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.

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25. Use of a (poly)peptide as identified by the method of claim 20 or of an agent as identified by the method of claim 21 or 22 for the preparation of a pharmaceutical composition for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including metabolic syndrome, obesity, and/or diabetes as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.
26. Use of a nucleic acid molecule as defined in any of claims 1-6 or 10 for the preparation of a medicament for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including obesity, diabetes, and/or metabolic syndrome, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.
27. Use of a polypeptide as defined in any one of claims 1 to 6, 8 or 9 for the preparation of a medicament for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including obesity, diabetes, and/or metabolic syndrome, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.
28. Use of a vector as defined in claim 7 or the preparation of a medicament for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including obesity, diabetes, and/or metabolic syndrome, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.

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29. Use of a host cell as defined in claim 18 or 19 for the preparation of a medicament for the treatment, alleviation and/or prevention of metabolic diseases or dysfunctions, including obesity, diabetes, and/or metabolic syndrome, as well as related disorders such as eating disorder, cachexia, hypertension, coronary heart disease, hypercholesterolemia, dyslipidemia, osteoarthritis, gallstones, or liver fibrosis.
30. Use of a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous nucleic acid molecule or of a functional fragment thereof for the production of a non-human transgenic animal which over- or under-expresses the foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous gene product.
31. Kit comprising at least one of
- (a) a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous nucleic acid molecule or a functional fragment thereof;
 - (b) a foraging, CG5535, CG14217, Klp67A, CG10133, CG3967, CG32048, CG13625, Baldspot, CG31605, fusilli, or schnurri homologous amino acid molecule or a functional fragment thereof;
 - (c) a vector comprising the nucleic acid of (a);
 - (d) a host cell comprising the nucleic acid of (a) or the vector of (c);
 - (e) a polypeptide encoded by the nucleic acid of (a);
 - (f) a fusion polypeptide encoded by the nucleic acid of (a);
 - (g) an antibody, an aptamer or another modulator/effector of the nucleic acid of (a) or the polypeptide of (b), (e), or (f) and

- 105 -

(h) an anti-sense oligonucleotide of the nucleic acid of (a).

Figure 1. Triglyceride content of *Drosophila foraging* (*for*; GadFly Accession Number CG10033) mutants

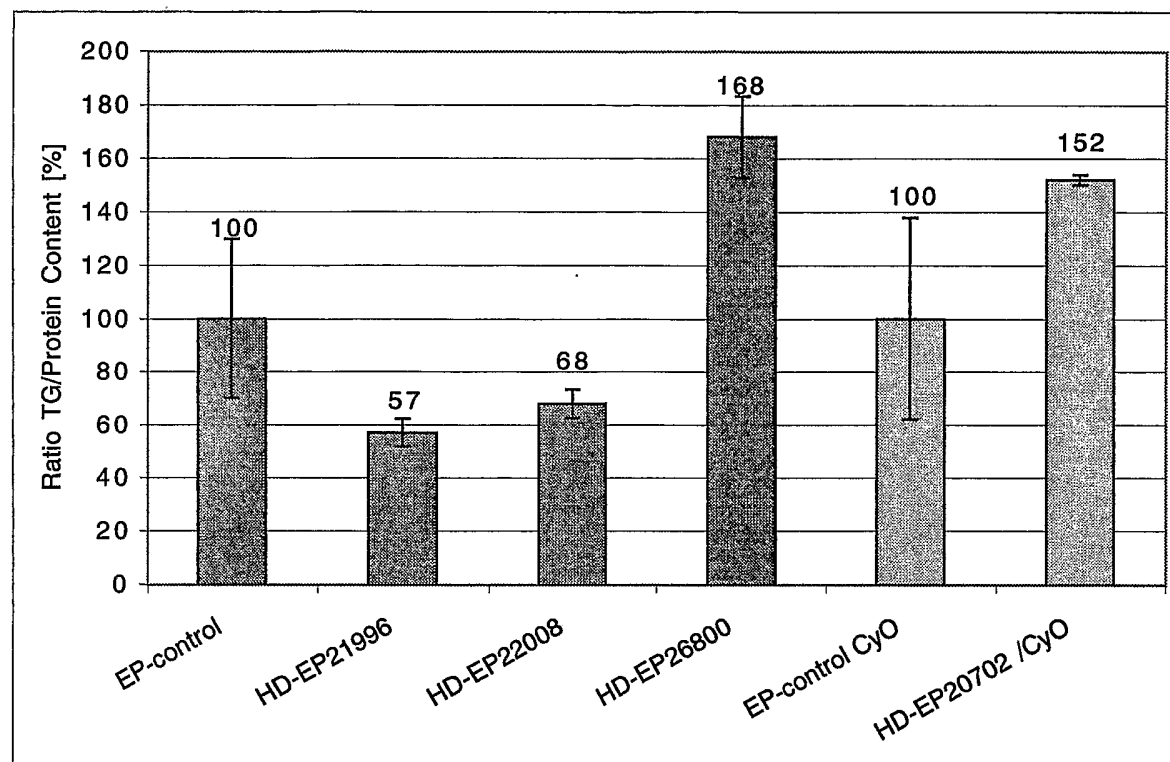


Figure 2. Molecular organisation of the *foraging* gene (GadFly Accession Number CG10033)

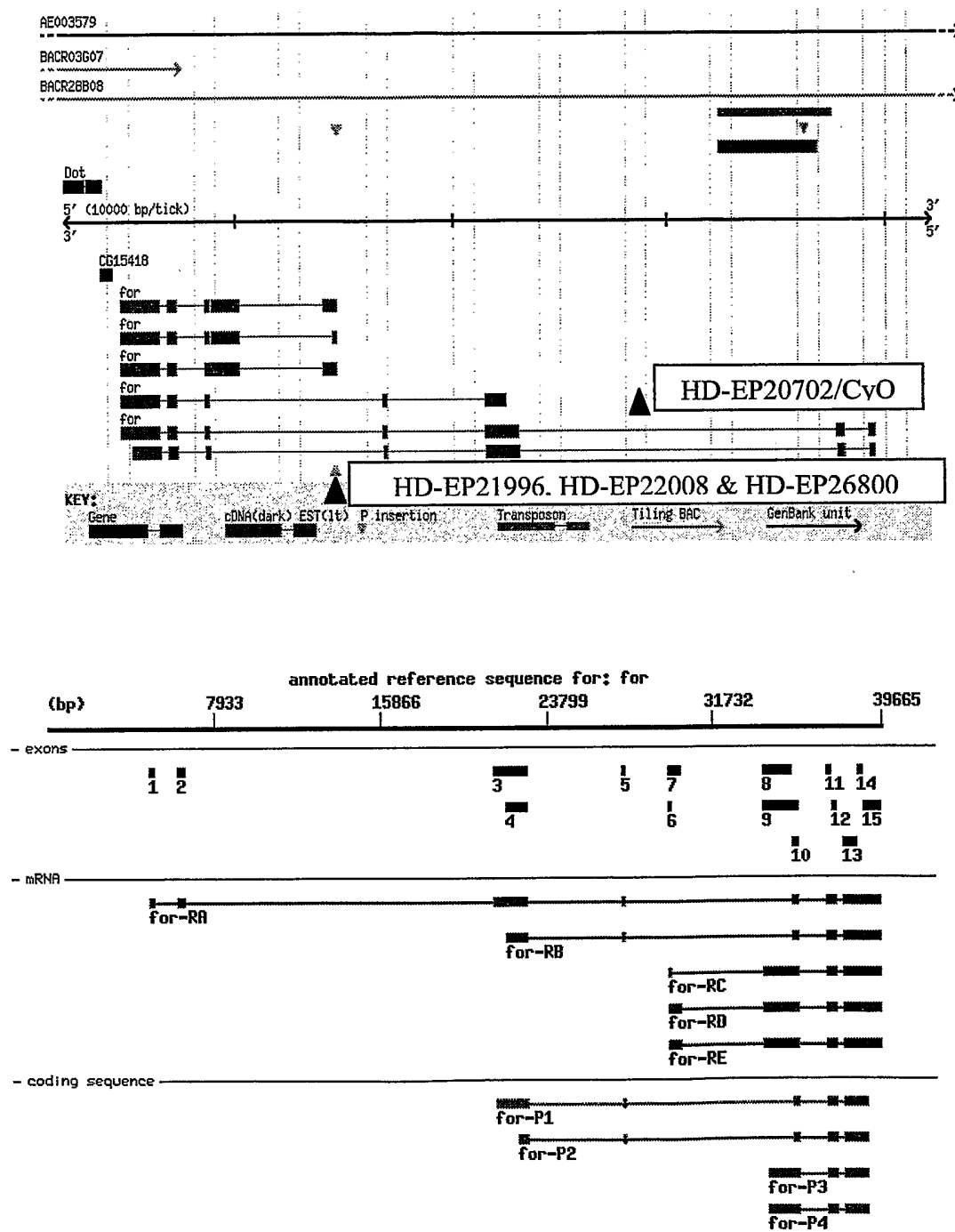


Figure 3. BLASTP results for *foraging* (GenBank Accession Number NP_477487)**Homology to human protein NP_006249.1 (GenBank Accession Number)**

gi|10835242|ref|NP_006249.1| (NM_006258) protein kinase, cGMP-dependent, type I;
Protein kinase, cGMP-dependent, regulatory, type I; cGKI; protein kinase, cGMP-
dependent, regulatory, type I, beta [Homo sapiens]; Length = 686

Score = 867 bits (2239), Expect = 0.0

Identities = 419/702 (59%), Positives = 529/702 (74%), Gaps = 35/702 (4%)

```

Query: 392  IQIIQAKELKIQEMQRALQFKDNEIAELKSHLDKFQSVFPFSRGSAAAGCAGTGGASGSGA 451
          I+ ++ ++ I E++ L KD I +L++ LDK++SV
Sbjct: 15   IEELRQRDALIDELELELDQKDELQKLNELDKYRSVIR----- 54

Query: 452  GSGSGSGPGTATGATRKSGQNFQ---RQALGISAEPQSESSLLLEHVSFPKYDKDERSR 508
          P T A ++S Q R + ISAEP + L HV+ P Y K +S+
Sbjct: 55  -----PATQQ-AQKQSASTLQGEPRTRQAI SAEPTAFDIQDLSHVTLPFFYPKSPQSK 106

Query: 509  ELIKAAILDNDFMKNLDLTQIREIVDCMYPVKYPAKNLIIEKGDVGSIVYVMEDGRVEVS 568
          +LIK AILDNDFMKNL+L+QI+EIVDCMYPV+Y + IIEKGDVGS+VYVMEDG+VEV+
Sbjct: 107  DLIKEAILDNDFMKNLELSQIQEIVDCMYPVEYKGDSCIIIEKGDVGSIVYVMEDGKVEVT 166

Query: 569  REGKYLSTLSGAKVLGELAILYNCQRTATITATECNLWAIERQCFQTIMMRTGLIRQAE 628
          +EG L T+ KV GELAILYNC RTAT+ + LWAI+RQCFQTIMMRTGLI+ E
Sbjct: 167  KEGVKLCTMGPGKVFGELAILYNCRTATVKTTLNVNKLWAI DRQCFQTIMMRTGLIKHTE 226

Query: 629  YSDFLKSVPFVKDLAEDTLIKISDVLEETHYQRGDYIVRQGARGDTFFIISKGVKRVVTIK 688
          Y +FLKSVP F+ L E+ L K++DVLEETHY+ G+YI+RQGARGDTFFIISKG V VT +
Sbjct: 227  YMEFLKSVPFTQSLPEEILSKLADVLEETHYENGEYIIRQGARGDTFFIISKGTVNVTRE 286

Query: 689  QQDTQEEKFIRMLGKGDFFGEKALQGDDLRTANIICESADGVSCLVIDRETFNQLISNLD 748
          +++ F+R LGKGD+FGKALQG+D+RTAN+I +A+ V+CLVIDR++F LI LD
Sbjct: 287  DSPSEDPVFLRTLGLKGDWFGKALQGEDVVRTANVI--AAEAVTCLVIDRDSFKHLIGGLD 344

Query: 749  EIKHR-YDDEGAMERRKINEE-FRDINLTDLRVIAITLGVGGFGRVELVQTNGDSSRSFAL 806
          ++ ++ Y+D A + + F ++ L+D +I TLGVGGFGRVELVQ + S++FA+
Sbjct: 345  DVSNNKAYEDAEAKAKYEAEAAFFANLKLSDFNIIIDTLGVGGFGRVELVQLKSEESKTFAM 404

Query: 807  KQMKKSQIVETRQQQHIMSEKEIMGEANCQFIVKLFKTFKDKKYLMLMESCLGGELWTI 866
          K +KK IV+TRQQ+HI SEK+IM A+ FIV+L++TFKD KYLYMLME+CLGGELWTI
Sbjct: 405  KILKKRHIVDTRQQEHIRSEKQIMQGAHSDFIVRLYRTFKDSKYLYMLMEACLGGELWTI 464

Query: 867  LRDKGNFDDSTTRFYTACVVEAFDYLSHRNIIYRDLKPENLLLNERGYVKLVDFGFACKL 926
          LRD+G+F+DSTTRFYTACVVEAF YLHS+ IYRDLKPENL+L+ RGY KLVDFGFACK+
Sbjct: 465  LRDRGSFEDSTTRFYTACVVEAFAYLHSGI IYRDLKPENLILDRGYAKLVDFGFACKI 524

Query: 927  QTGRKTWTFCTPEYVAPEVILNRGHDISADYWSLGVLMFELLTGTPPFTGSDPMRTYNI 986
          G+KTWTFCTPEYVAPE+ILN+GHDISADYWSLG+LM+ELLTG+PPF+G DPM+TYNI
Sbjct: 525  GFGKKTWTFCTPEYVAPEIILNKGHDISADYWSLGILMYELLTGSPPFSGPDPMKTYNI 584

Query: 987  ILKGIDAIEFPRNITRNASNLIKKLCRDNPAERLGYQRGGISEIQKHKWFDGFYWGLQN 1046
          IL+GID IEFP+ I +NA+NLIKKLCRDNP+ERLG + G+ +IQKHKWF+GF W GL+
Sbjct: 585  ILRGIDMIEFPKIAKNAANLIIKKLCRDNP SERLGNLKNVVKDIQKHKWFE GFNWGLRK 644

Query: 1047 CTLEPPIKPAVKSVDTTNFDDYPPDPEGPPDDVTGWDKDF 1088
          TL PPI P+V S DT+NFD +P D + PPPDD +GWD DF
Sbjct: 645  GTLTPIIPSVASPTDTSNFDSPEDNDEPPDDNSGWDIDF 686

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Homology to human protein NP_006250.1 (GenBank Accession Number)

gi|5453978|ref|NP_006250.1| (NM_006259) protein kinase, cGMP-dependent, type II; cGKII
[Homo sapiens]; Length = 762

Score = 664 bits (1713), Expect = 0.0

Identities = 346/739 (46%), Positives = 478/739 (63%), Gaps = 56/739 (7%)

```

Query: 401  KIQEMQRALQFKDNEIAELKSHLDKFSVFPFSRGSAAAGCAGTGGASGSG----- 450
             K+ E++R L+ KD EI E + HL + + S+ + A T
Sbjct: 29  KVTELERELRRKDAEIQEREYHLKELRE--QLSKQTVAIAELTEELQNKCIQLNKLQDVV 86

Query: 451  --AGGS--GGSGPGTATGATRKSGQ----NFQRQRALGISAEPPQSESSLLLEHVSF---- 498
             GGS S RK+ + +R G+SAEP + + L + F
Sbjct: 87  HMQGGSPQLQASPDKVPLEVHRKTSGLVSLHSRRGAKAGVSAEPTTRTYDLNKPPEFSFEK 146

Query: 499  PKYDKDERSRELKAAILDNDFMKNLDDLTLQIREIVDCMYPVKYPAKNLIKEGVDGSIIV 558
             + KD ++LI A+ N F+K LD QI+++V+CMY Y + IIK+G+ G+ ++
Sbjct: 147  ARVRKDSSEKKLITDALNKNQFLKRLDPQIKDMVECMYGRNYQQGSYIIKQGEPEGNHIF 206

Query: 559  VMEDGRVEVSREGKYLSTLSGAKVLGELAILYNLCQRTATITAITECNLWAIERQCFQTIM 618
             V+ +GR+EV + K LS++ GELAILYNLC RTA++ AIT WA++R+ FQ IM
Sbjct: 207  VLAEGRLLEVFGQEKLLSSIPMWTTFGELAILYNLCRTASVKAITNVKTVWALDREVFNQIM 266

Query: 619  MRTGLIRQAEYSDFLKSVPFIKDLAEDTLIKISDVLEETHYQRGDYIVRQGARGDTFFII 678
             RT R +Y +FL+SV + K+L ED L KI D LE +Y +GDYI+R+G G TFFI+
Sbjct: 267  RRTAQARDEQYRNFLRSVSLNLPEDKLTKIIDCLEVEYYDKGDYIIREGEEGSTFFIIL 326

Query: 679  SKGKVRVTIKQQDTQEEKFIRMLGKGDFGGEKALQGDDLRTANIICESADGVSCLVIDRE 738
             +KGKV+VT + + + I+ L KG++FGEKAL DD+R+ANII E D V+CLVIDRE
Sbjct: 327  AKGKVKVTQSTEGHDQPQLIKTLQKGEYFGEKALISDDVRSANIIAEEND-VACLVIDRE 385

Query: 739  TFNQLISNLDEIKH-----RYDDEGAMERRKINE-----EFRDINLTD----- 776
             TFNQ + +E++ DDE +R ++ I L +
Sbjct: 386  TFNQTVGTFEELQKYLEGYVANLNRDDEKRHAKRSMNSNWKLSKALSLEMIQLKEKVARFS 445

Query: 777  -----LRVIATLGVGGFGRVELVQTNQDSSRSFALKQMKKSQIVETRQQQHIMSEKEI 829
             L +IATLGVGGFGRVELV+ ++ +FA+K ++K IV+T+QQ+H+ SEK I
Sbjct: 446  SSSPFQNLLEIIATLGVGGFGRVELVVKVKNENV-AFAMKCIRKKHIVDTKQQEHVYSEKRI 504

Query: 830  MGEANCQFIVKLKFTFKDKKLYMLMESCLGGELWTLIRDKGNFDDSTTRFYTACVVEAF 889
             + E FIVKL++TFKD KY+YML+E+CLGGELW+ILRD+G+FD+ T++F ACV EAF
Sbjct: 505  LEELCSPFIVKLYRTFKDNKYVYMLLEACLGELWSILRDRGSFDEPTSKFCVACVTEAF 564

Query: 890  DYLSHRNI IYRDLKPENLLLNERGYVKLVDFGFAGKLQGRKTWTFCGTPEYVAPEVILN 949
             DYLN IYRDLKPENL+L+ GY+KLVDGFGAKK+ +G+KTWTFCGTPEYVAPEVILN
Sbjct: 565  DYLSHRLGI IYRDLKPENLILDAEGYLKLVDFGFAGKIGSGQKTWTFCGTPEYVAPEVILN 624

Query: 950  RGHDISADYWSLGVLMFELLTGTPPFTGSDPMRTYNIILKGIDAIEFPRNITRNASNLIK 1009
             +GHD S D+WSLG+L++ELLTG PPF+G D M TYN+ILKGI+ ++FPR ITR +LI+
Sbjct: 625  KGHDFSVDFWSLGILVYELLTGNPPFSGVDQMMTYNLIILKGIKMDFPKIRTRRPEDLIR 684

Query: 1010 KLCRDNPAERLGYQRGGISEIQKHKWFDFGYWGLQNCTLEPPIKPAVKSVDTTNFDDY 1069
             +LCR NP ERLG + GI++I+KH+W +GF W GL+ +L P++ +K +D + FD Y
Sbjct: 685  RLCRQNPTERLGNLKNKGINDIKKHRLWNGFNWEGLKARSLPSPLQRELKGPIDHSYFDKY 744

Query: 1070 PPDPEGPPDDVTGWKDF 1088
             PP+ +G PPD+++GWKDF
Sbjct: 745  PPE-KGMPPDELSGWKDF 762

```

Figure 4. Expression of *foraging* Homologs in Mammalian Tissues

Figure 4A. Real-time PCR analysis of protein kinase, type I (Prkg1) expression in wild type mouse tissues

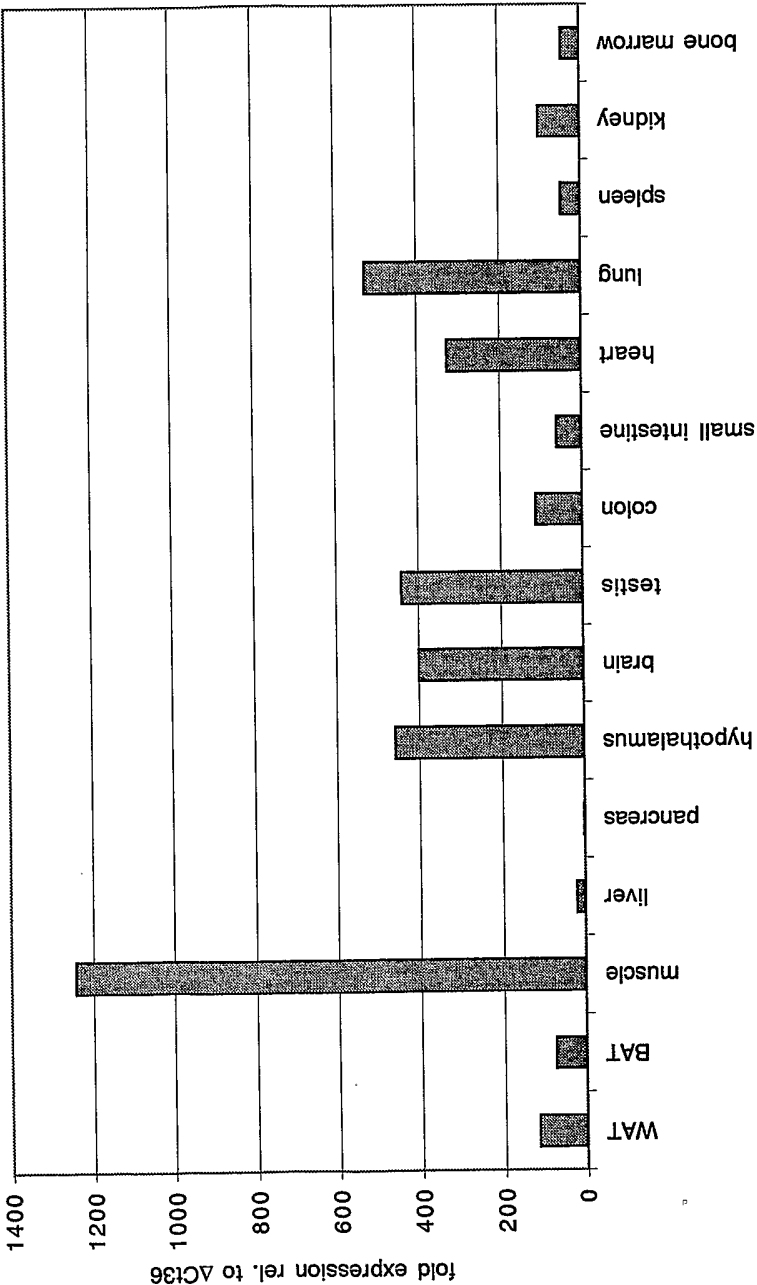


Figure 4B. Real-time PCR analysis of Prkg1 expression in different mouse models

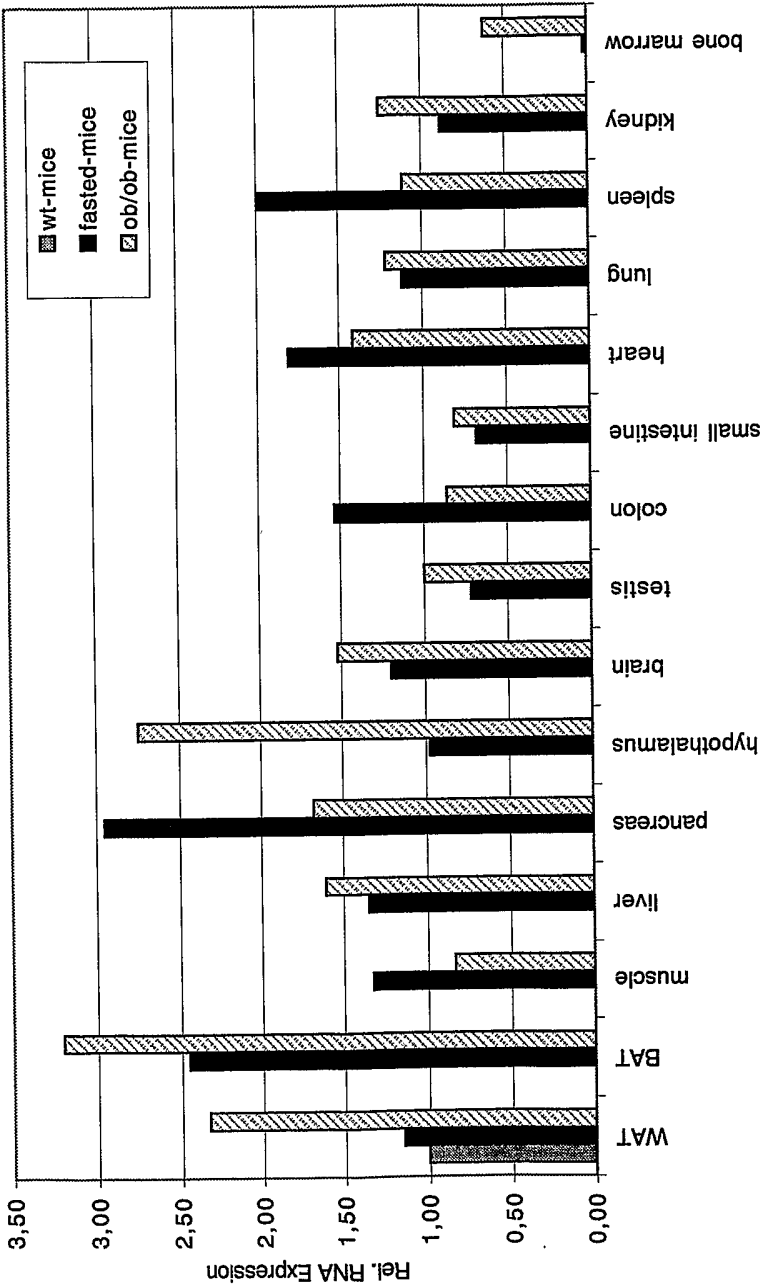


Figure 4C. Real-time PCR analysis of Prkg1 expression in mice fed with a high fat diet compared to mice fed with a standard diet

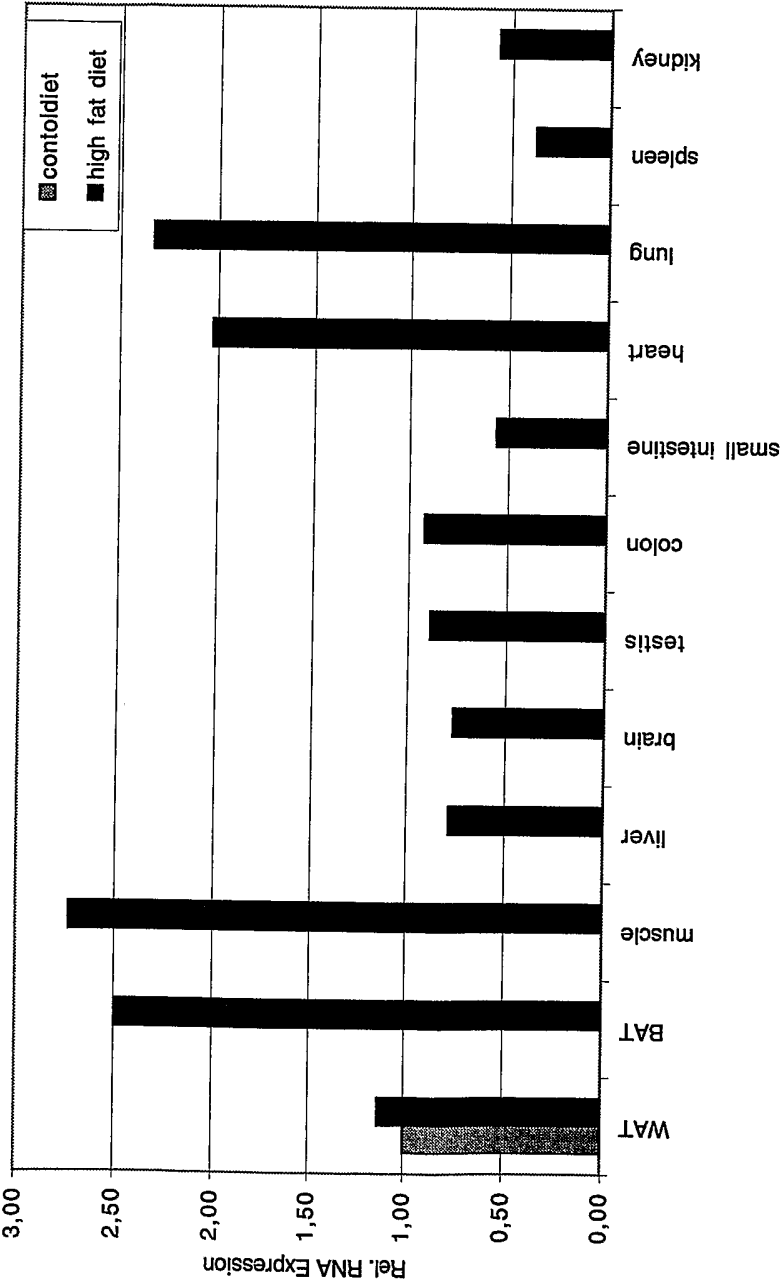


Figure 4D. Real-time PCR analysis of Prkg1 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

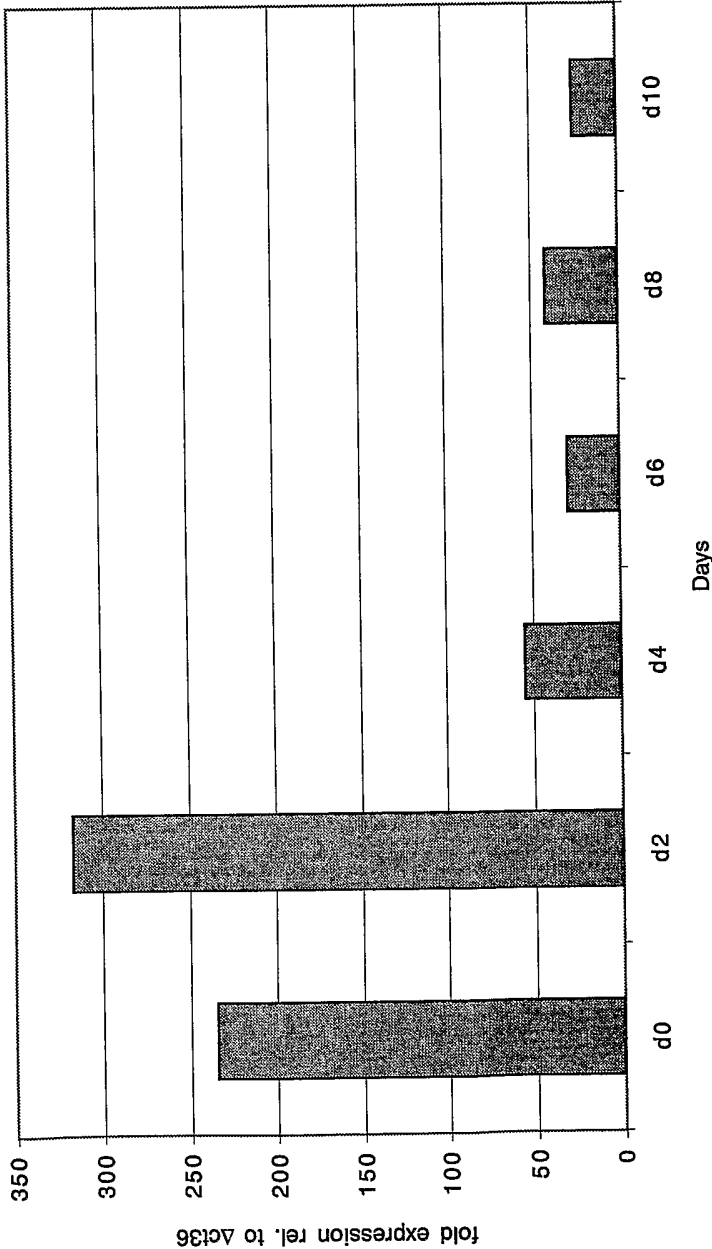


Figure 4E. Real-time PCR analysis of protein kinase, cGMP-dependent, type II (Prkg2) expression in wild type mouse tissues

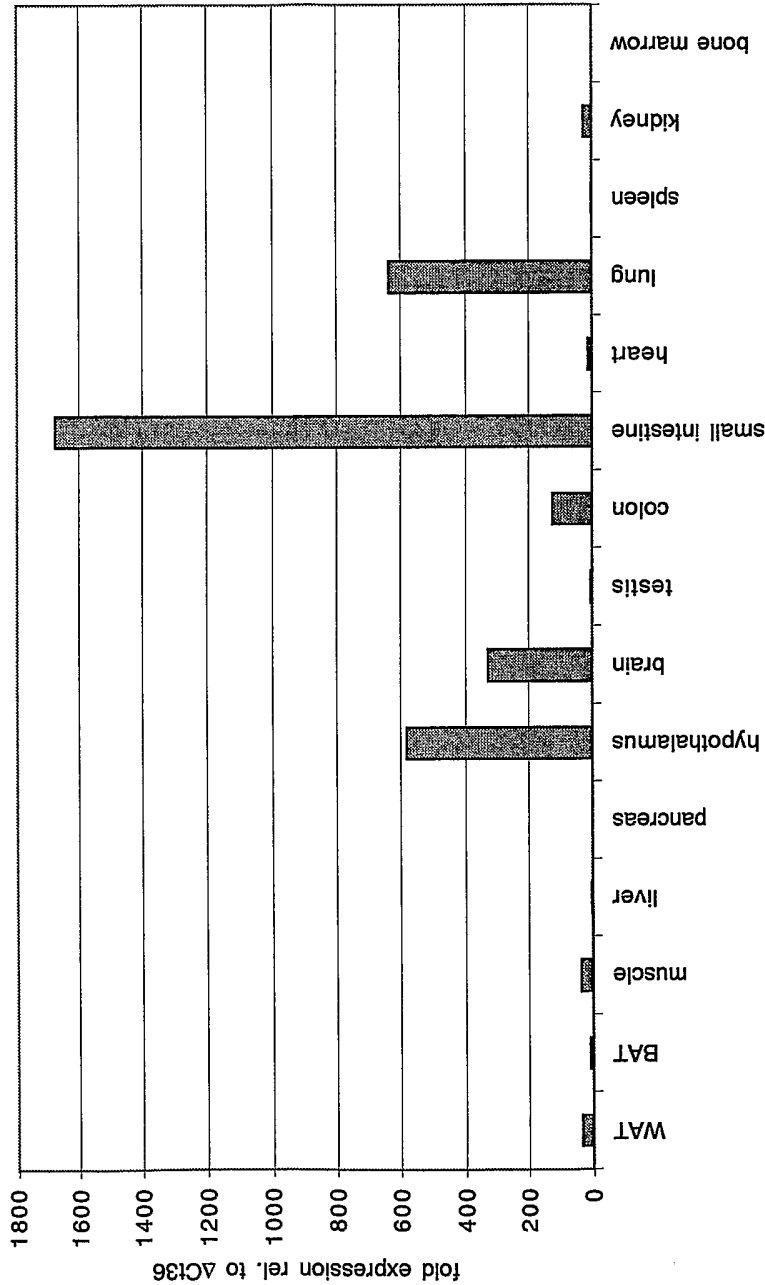


Figure 4F. Real-time PCR analysis of Prkg2 expression in different mouse models

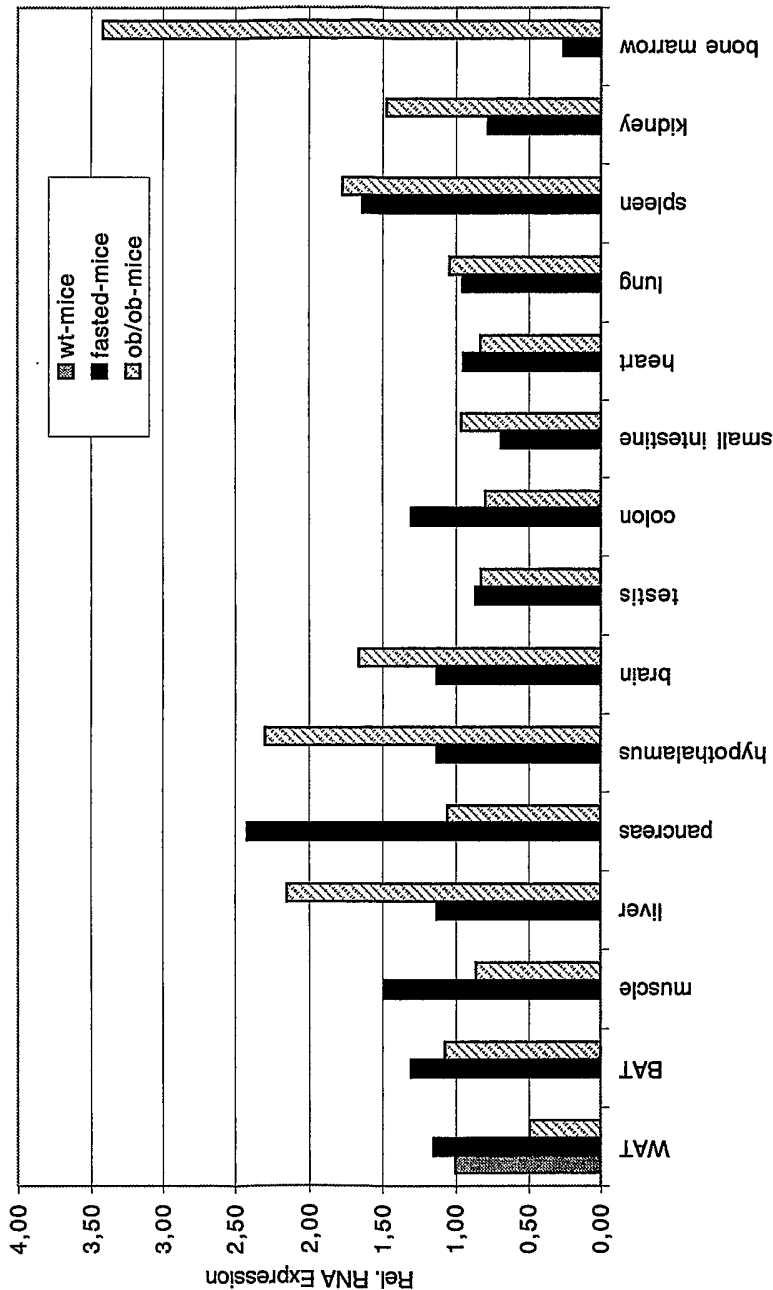


Figure 4G. Real-time PCR analysis of Prkg2 expression in mice fed with a high fat diet compared to mice fed with a standard diet

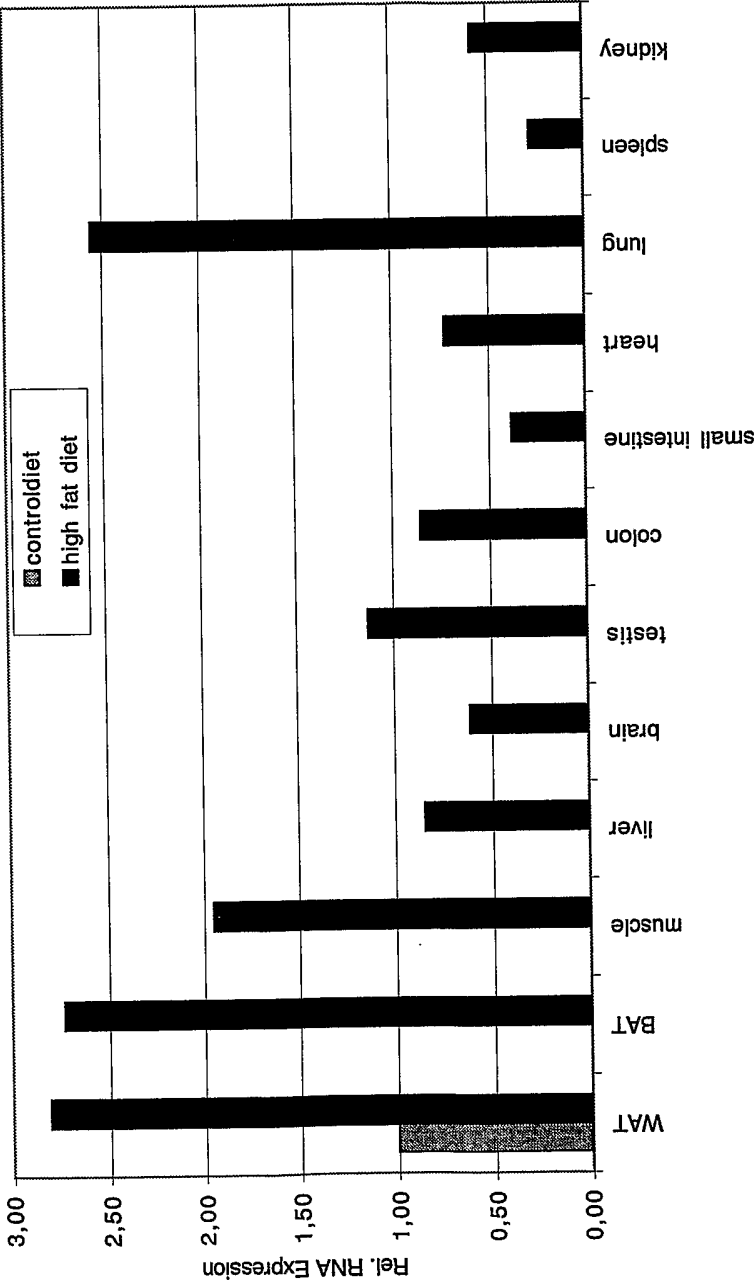


Figure 4H. Real-time PCR analysis of Prkg2 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

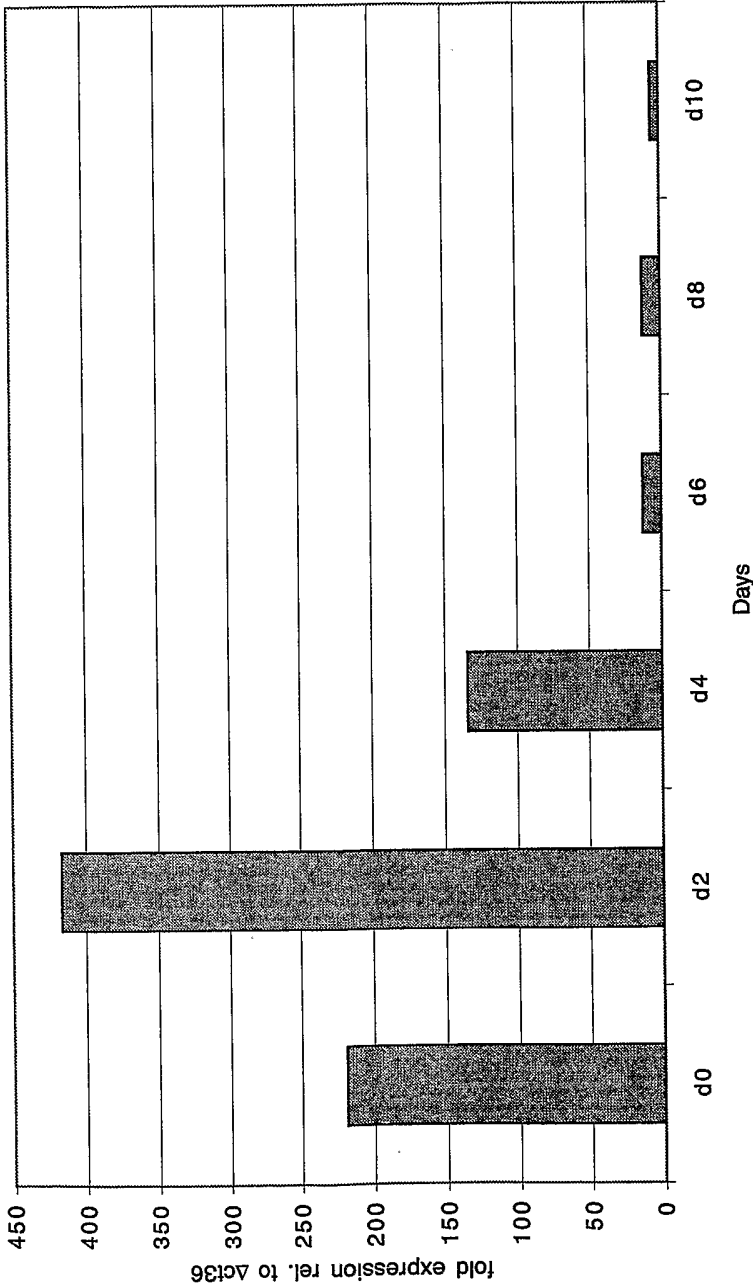


Figure 5. Expression of a human *foraging* homolog in mammalian (human) tissue. Quantitative analysis of protein kinase, cGMP-dependent, type II (PRKG2) expression in human abdominal adipocyte cells during the differentiation from preadipocytes to mature adipocytes

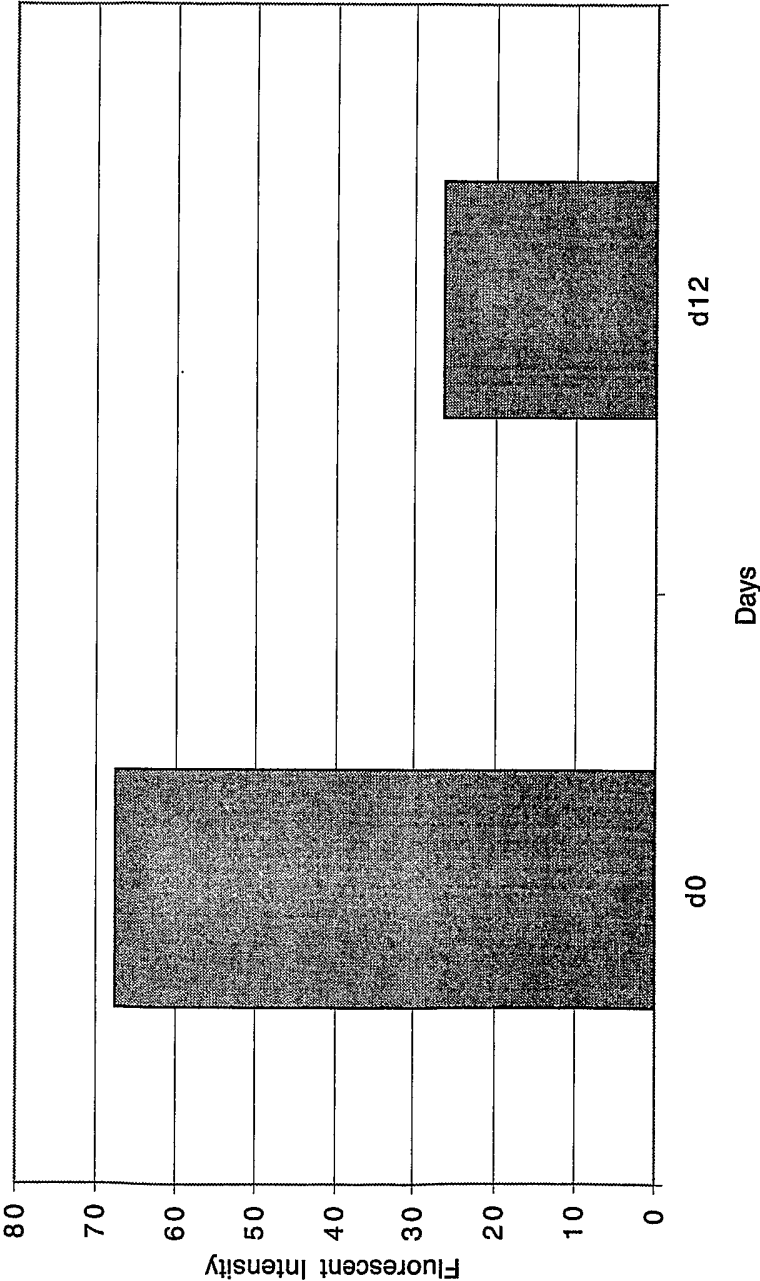


Figure 6. Triglyceride content of a *Drosophila* cationic amino acid transporter (GadFly Accession Number CG5535) mutant

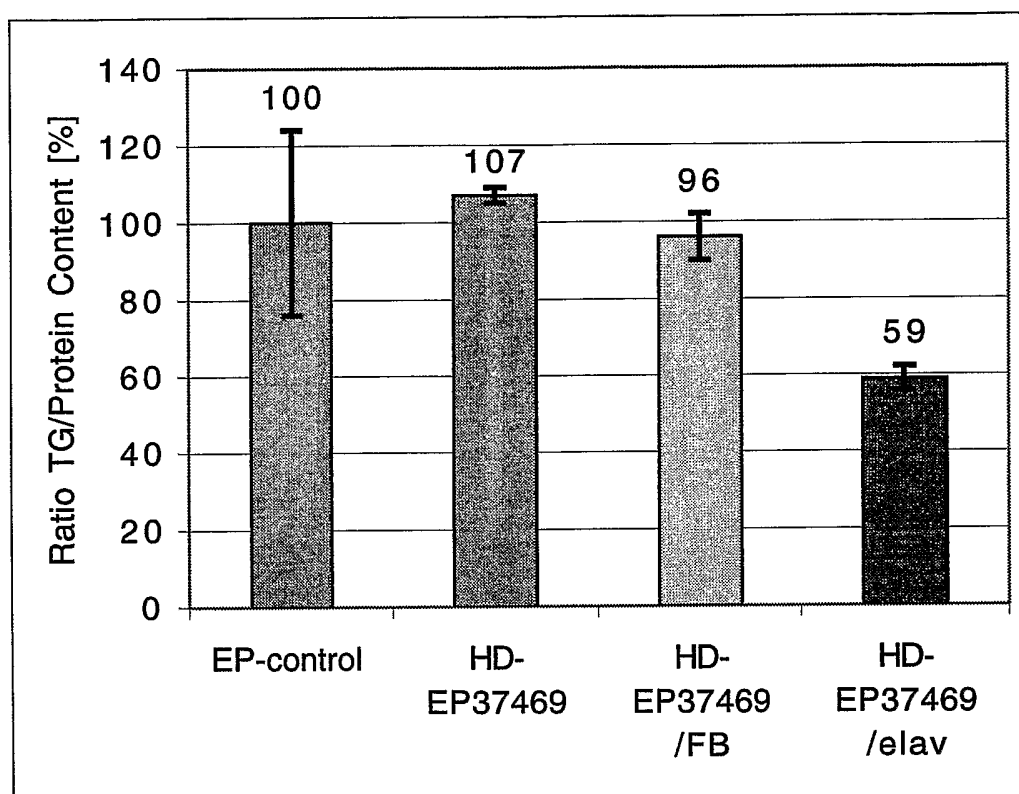


Figure 7. Molecular organization of the cationic amino acid transporter gene (GadFly Accession Number CG5535)

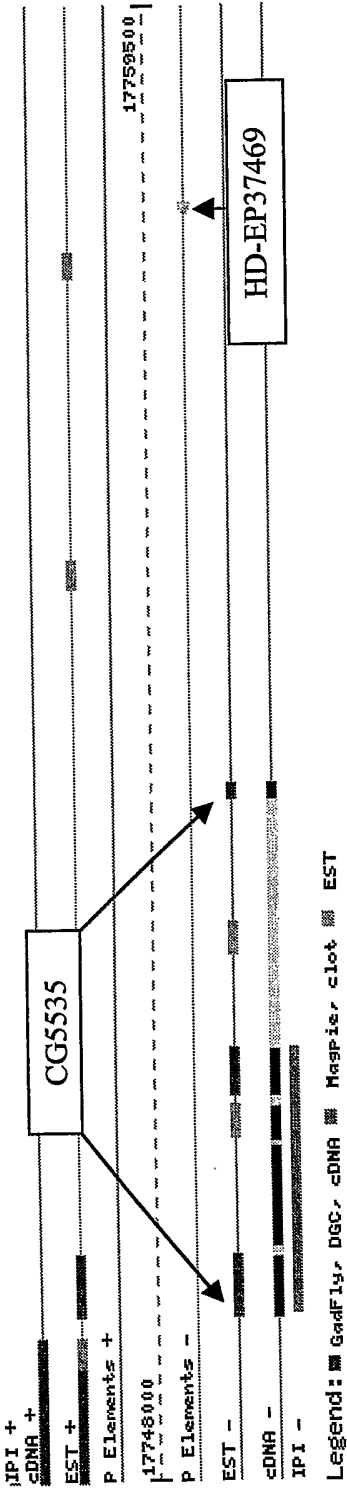


Figure 8. BLASTP results for cationic amino acid transporter (GadFly Accession Number CG5535)

Homology to human protein NP_003037.1 (GenBank Accession Number)

ref|NP_003037.1| (NM_003046) solute carrier family 7 (cationic amino acid transporter, y+ system), member 2; Solute carrier family 7 (cationic amino acid transporter, y+ system),; amino acid transporter, cationic 2 [Homo sapiens]
Length = 657

Score = 530 bits (1365), Expect = e-149

Identities = 275/605 (45%), Positives = 391/605 (64%), Gaps = 30/605 (4%)

```

Query: 23  RVLTRKK--PLEDSNESKLAKVLSAFDLTALGIGSTLGVGVVVLAGEVSKQYAGPAVVVS 80
          R L R+K  L+  ++KL + LS DL ALG+GSTLG GVVVLAGEV+K  +GP++VVS
Sbjct: 12  RCLIRRKIVTLDLEDTKLCRCLSTMDLIALGVGSTLGAGVVVLAGEVAKADSGPSIVVS 71

Query: 81  FLIAAIASIFAGLCYAEFGARVPKAGSAYIYSYVTIGEFIAFLIGWNLILEYAIGSASVV 140
          FLIAA+AS+ AGLCYAEFGARVPK GSAY+Y+YVT+GE AF+ GWNLIL Y IG++SV
Sbjct: 72  FLIAALASVMAGLCYAEFGARVPKTSAYLYTYVTVGELWAFITGWNLILSYVIGTSSVA 131

Query: 141 KGLSTYLDQLCGNPMSSFLGTHMPLNIEGMGAYPDLFAFVVTILFSLAIAVGAKESTRVN 200
          + S D+L  + FL T+ +N G+ YPD FA + +L + ++ G KES VN
Sbjct: 132 RAWSGTFDELLSKQIGQFLRTYFRMNYTGLAEYPDFFAVCLILLLAGLLSFGVKESAWVN 191

Query: 201 NVFTMLNLGVVLFVIIAGLFKVSSSNWSI-----PKSQVPEGYGDGGFMP 245
          VFT +N+ V+LFV++AG K + +NW I P YG GGFMP
Sbjct: 192 KVFTAVNILVLLFVMVAGFVKGNVANWKISEEFLKNISASAREPPSENGTSTYAGGGFMP 251

Query: 246 YGVSGIIGKAAVCFYGFYGFDCIATAGEEAKNPKKSIPFAVIVSLAMIFLAYFGVSTVLT 305
          YG +G + GAA CFY F+GFDCIAT GEE +NP+K+IP ++ SL + F+AYFGVS LT
Sbjct: 252 YGFTGTLAGAATCFYAFVGFDCIATTGEEVRNPQKAIPIGIVTSLLVCFMAYFGVSAALT 311

Query: 306 MMLPYFEQDEKAPLPHVFRINGWHVAEYVVSIGAMFGLCSSMMGAMFPLPRIVFAMSNDG 365
          +M+PY+ DEK+PLP F GW A+YVV+ G++ L +S++G+MFPLPRI+FAM+ DG
Sbjct: 312 LMPYLYLDEKSPLPVAFEVVGWGPAYVVAAGSLCALSTSLGSMFPLPRILFAMARDG 371

Query: 366 LLFKFLGDISKEYKTPFKGTMITGMLTGILAAVFNLSQLVNMMSIGTLLAYSMVASCVLM 425
          LLF+FL +S K ++P T+ G+++ ++A +F+L LV+MMSIGTL+AYS+VA+CVL+
Sbjct: 372 LLFRFLARVS-KRQSPVAATLTAGVISALMAFLFDLALVDMMSIGTLMAYSLVAACVLI 430

Query: 426 LRYEVD-----DRRESRIVANGRATGLEQDRPCALWRRIFNLN----GQTVPTKQTS 473
          LRY+ + + ++ R T + + L R+ F++ +PT+Q++
Sbjct: 431 LRYQPGLSYDQPKCSPEKDGLGSSPRVTSKSESQVTMLQRQGFMRTLFCPSLLPTQQSA 490

Query: 474 RIVTYSVTFLFSLWCMVFSQILTKFEEDLANVTSFDGIKLVLTGTLPLAVLLLVISRQPTSG 533
          +V++ V + + S + T + + ++ L L + ++L I RQP +
Sbjct: 491 SLVSFLVGFLAFLVLGLSVLTYYGVHAI TRLEAWSLALLTLFLVLFVAIVLTIWRQPQNQ 550

Query: 534 VKLSFKVPLVPWLPGISILINIYLMIKLDILTWIRFSIWIAIGLAIFLAYGIRHSRLRQR 593
          K++F VP +P+LP SIL+NIYLM++L TW+RFSIW+AIG I+ +YGIRHS
Sbjct: 551 QKVAFMVPFLPFLPAFSILVNIYLMVQLSADTWVRFISIWMAIGFLIYFSYGIRHSLEGHL 610

Query: 594 EQRNN 598
          NN
Sbjct: 611 RDENN 615

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Homology to human protein NP_003036.1 (GenBank Accession Number)

ref|NP_003036.1| (NM_003045) solute carrier family 7 (cationic amino acid transporter, y+ system), member 1; ecotropic retroviral receptor; Solute carrier family 7 (cationic amino acid transporter, y+ system),; amino acid transporter, cationic 1 [Homo sapiens]
Length = 629

Score = 517 bits (1331), Expect = e-145

Identities = 268/602 (44%), Positives = 389/602 (64%), Gaps = 34/602 (5%)

Query: 20 NAFRVLTRKKPLEDSNE-SKLAKVLSAFDLTALGIGSTLGVGVVLAGEVSKQYAGPAVV 78
N + + R+K ++ S E ++L++ L+ FDL ALG+GSTLG GVVVL V+++ AGPA+V
Sbjct: 8 NIGQQMLRRKVVDVDCSREETRLSRCLNTFDLVALGVSTLGAGVVVLAVARENAGPAIV 67

Query: 79 VSFLIAAIAISIFAGLCYAEFGARVPKAGSAYIYSYVTIGEFIAFLIGWNLILEYAIGSAS 138
+SFLIAA+AS+ AGLCY EFGARVPK GSAY+YSYVT+GE AF+ GWNLIL Y IG++S
Sbjct: 68 ISFLIAALASVLAGLCYGEFGARVPKTSAYLYSYVTVGELWAFITGWNLILSYIIGTSS 127

Query: 139 VVKGLSTYLDQLCGNPMSSFLGTHMPLNIEGMGAY-PDLFAFVVTLFSLAIAVGAKEST 197
V + S D+L G P+ F THM LN G+ A PD+FA ++ ++ + + +G KES
Sbjct: 128 VARAWSATFDELIGRPIGEFSRTHMTLNAPGVLAENPDIFAVIILITGLTLGVKESA 187

Query: 198 RVNNVFTMLNLGVVLFVIIAGLFKVSSSNWSIPK-----SQVPEGY-GDGGF 243
VN +FT +N+ V+ F++++G K S NW + + + EG G GGF
Sbjct: 188 MVNKIFTCINVLVLGFMVSGFVKGSVKNWQLTEEDFGNTSGRLCLNNDTKGKPGVGGF 247

Query: 244 MPYGVSGIIKGAAVCFYGFIFDCIATAGEEAKNPKKSIPFAVIVSLAMIFLAYFGVSTV 303
MP+G SG++ GAA CFY F+GFDCIAT GEE KNP+K+IP ++ SL + F+AYFGVS
Sbjct: 248 MPFGFSGVLGAATCFYAFVGFDCIATTGEEVKNPQKAIPVGIVASLLICFIAYFGVSAA 307

Query: 304 LTMMLPYFEQDEKAPLPHVFRINGWHVAEYVVSIGAMFGLCSSMMGAMFPLPRIVFAMSN 363
LT+M+PYF D +PLP F+ GW A+Y V++G++ L +S++G+MFP+PR+++AM+
Sbjct: 308 LTLMPYFCLDNNSPFLPDAFKHVGWEGAKYAVAVGSLCALSASLLGSMFPMPRVIYAMAE 367

Query: 364 DGLLFKFLGDISSEKYKTPFKGTMITGMLTGILAAVFNLSQLVNMMSIGTLLAYSMVASCV 423
DGLLFKFL +++++ KTP T+ +G + ++A +F+L LV++MSIGTLLAYS+VA+CV
Sbjct: 368 DGLLFKFLANVNDRTKTPIIATLASGAVAAVMAFLFDLKDLDLMSIGTLLAYSLVAACV 427

Query: 424 LMLRY-----EVDDRRESRIVA-NGRATGLEQDRPCALWRRIFNLNGQT 466
L+LRY E+D ++ + + N G + + I +
Sbjct: 428 LVLRYQPEQPNLVYQMASTSELDPADQNELASTNDSQLGFLPEAEMFSLKTILSPKNME 487

Query: 467 VPTKQTSRIVTYSVTLFSLWCMVFSQILTKFEEDLANVTSFDGIKLVLTPIPLAVLLVI 526
P+K + IV S +L ++ + F + E L + L + AV+ VI
Sbjct: 488 -PSKISGLIVNISTSLIAVLIITFCIVTVLGREALTKGALWAVFLLAGSALLCAVVTGVI 546

Query: 527 SRQPTSGVKLSFKVPLVPWLPGISILINIYLMIKLDILTWRFSIWIAIGLAIFLAYGIR 586
RQP S KLSFKVP +P LP +SI +N+YLM++LD TW+RF++W+ IG I+ YG+
Sbjct: 547 WRQPESKTKLSFKVPFLPVLPILSIFVNVYLMMLDQGTWVRFAVWMLIGFIIYFGYGLW 606

Query: 587 HS 588
HS
Sbjct: 607 HS 608

Homology to human protein NP_116192.2 (GenBank Accession Number)

ref|NP_116192.2| (NM_032803) solute carrier family 7 (cationic amino acid transporter, y+ system), member 3 [Homo sapiens]
Length = 619

Score = 504 bits (1297), Expect = e-141
Identities = 264/590 (44%), Positives = 387/590 (64%), Gaps = 20/590 (3%)

Query: 16 WSVTNAF-RVLTRKKPLEDSN-ESKLAKVLSAFDLTALGIGSTLGVGVVLAGEVSKQYA 73
W F + L R++ LE E++LA+ LS DL ALG+GSTLG GVVVLAGEV+K A
Sbjct: 3 WQAFRRFGQKLVRRRTLESGMAETRLARCLSTLDLVALGVGSTLGAGVVVLAGEVAKDKA 62

Query: 74 GPAVVVSFLIAAIASIFAGLCYAEFGARVPKAGSAYIYSYVTIGEFIAFLIGWNLILEYA 133
GP++V+ FL+AA++S+ AGLCYAEFGARVP++GSAY+YSYVT+GE AF GWNLI L Y
Sbjct: 63 GPSIVICFLVAALSSVLAGLCYAEFGARVPRSGSAYLYSYVTVGELWAF TTGWNLI LSYV 122

Query: 134 IGSASVVKGLSTYLDQLCGNPMSSFLGTHMPLNIEGMGA-YPDLFAFVVTILFSLAIAVG 192
IG+ASV + S+ D L GN +S L + L++ + A YPD FA + +L + +A+G
Sbjct: 123 IGTASVARAWSSAFDNLIGNHISKTLQGSIALHVPVHVAEYPDFFALGLVLLLTGLLALG 182

Query: 193 AKESTRVNNVFTMLNLGVVLFVIIAGLFKVSSSNWSIPK-----SQVPEGY-----GD 240
A ES V VFT +NL V+ FV+I+G K NW + + +++ + Y G
Sbjct: 183 ASESALVTKVFTGVNLLVLGFMISGFVKGDVHNWKLTEEDYELAMAE LNDTYSLGPLGS 242

Query: 241 GGFMPYGVSGIIGAAVCFYGFIFGDCIATAGEEAKNPKKSIPFAVIVSLAMIFLAYFGV 300
GGF+P+G GI++GAA CFY F+GFDCIAT GEEA+NP++SIP +++SL++ FLAYF V
Sbjct: 243 GGFVFPFGFEGILRGAATCFYAFVGFDCIATTGEEAQN PQRSIPMGIVISLSVCFLAYFAV 302

Query: 301 STVLTMMLPYFEQDEKAPLPHVFRINGWHVAEYVVSIGAMFGLCSSMMGAMFPLPRIVFA 360
S+ LT+M+PY++ ++PLP F GW A YV++G++ L +S++G+MFP+PR+++A
Sbjct: 303 SSALTLMMPYYQLQPESPLPEAFLYIGWAPARYVAVGSLCALSTSLLGSMFMPRVIYA 362

Query: 361 MSNDGLLFKFLGDISEKYKTPFKGTMITGMLTGILAAVFNLSQLVNMSIGTLLAYSMVA 420
M+ DGLLF+ L I +TP T+++G++ +A +F L+ LV++MSIGTLLAYS+V+
Sbjct: 363 MAEDGLLFRVLARIHTGTRTPIIATFVSGIIAFAFMAFLFKLTDLVDLMSIGTLLAYSLVS 422

Query: 421 SCVLMRLRYEVD DRRES--RIVANGRATGLEQDRPCALWRRIFNLNGQTVPTKQTSRIVTY 478
CVL+LRY+ D ++ + A E ++ LW F LN ++PT + +IV
Sbjct: 423 ICVLILRYQPQETKTGEEVELQEEAITTESEK-LTLWGLFFPLN--SIPTPLSGQIVYV 479

Query: 479 SVTLFSLWCMVFSQILTKFEEDLANVTSFSGIKLVLTGTLPLAVLLLVISRQPTSGVKLSF 538
+L ++ +L ++ L + +VL + + +++VI RQP S L F
Sbjct: 480 CSSLLAVLLTALCLVLAQWSVPLLSGDLWTAVVLLLLLLIIGIIVVIWRQPQSSTPLHF 539

Query: 539 KVP LVPWLPGISILINIYLMIKLDILT WIRFSIWIAIGLAIFLAYGIRHS 588
KVP +P LP +SI +NIYLM+++ TW RF +W+ IG AI+ YGI+HS
Sbjct: 540 KVPALPLPLMSIFVNIYLMQM TAGTWARFGVWMLIGFAIYFGYGIQHS 589

Figure 9. Expression of CG5535 Homologs in Mammalian Tissues

Figure 9A. Real-time PCR analysis of solute carrier family 7, member 1 (Slc7a1) expression in wild type mouse tissues

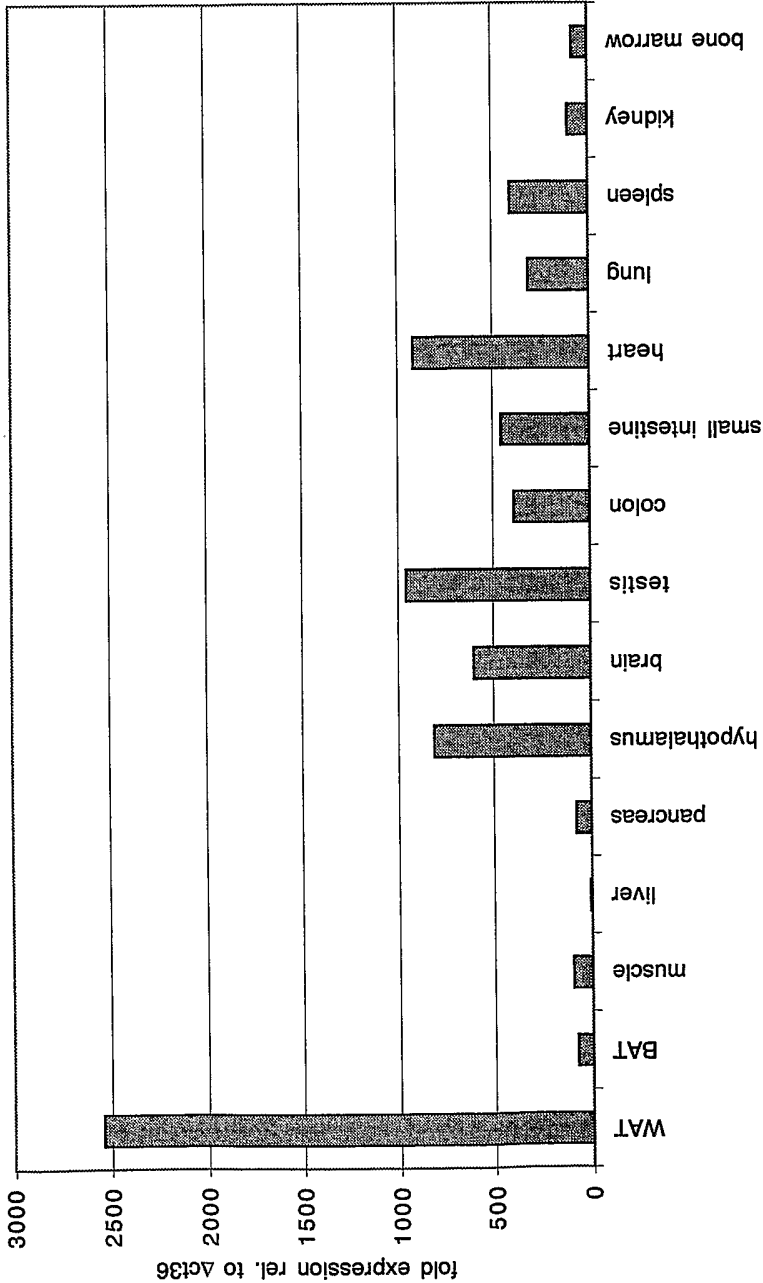


Figure 9B. Real-time PCR analysis of Slc7a1 expression in different mouse models

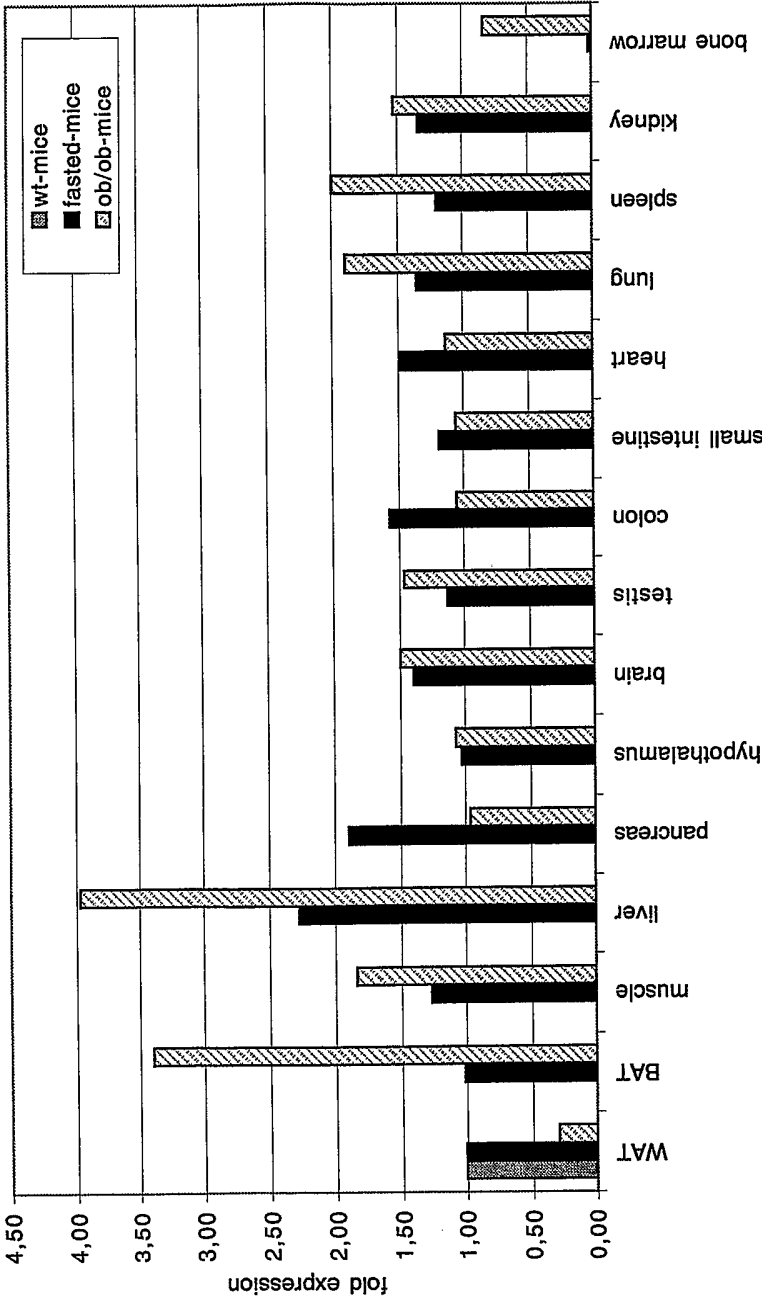


Figure 9C. Real-time PCR analysis of Slc7a1 expression in mice fed with a high fat diet compared to mice fed with a standard diet

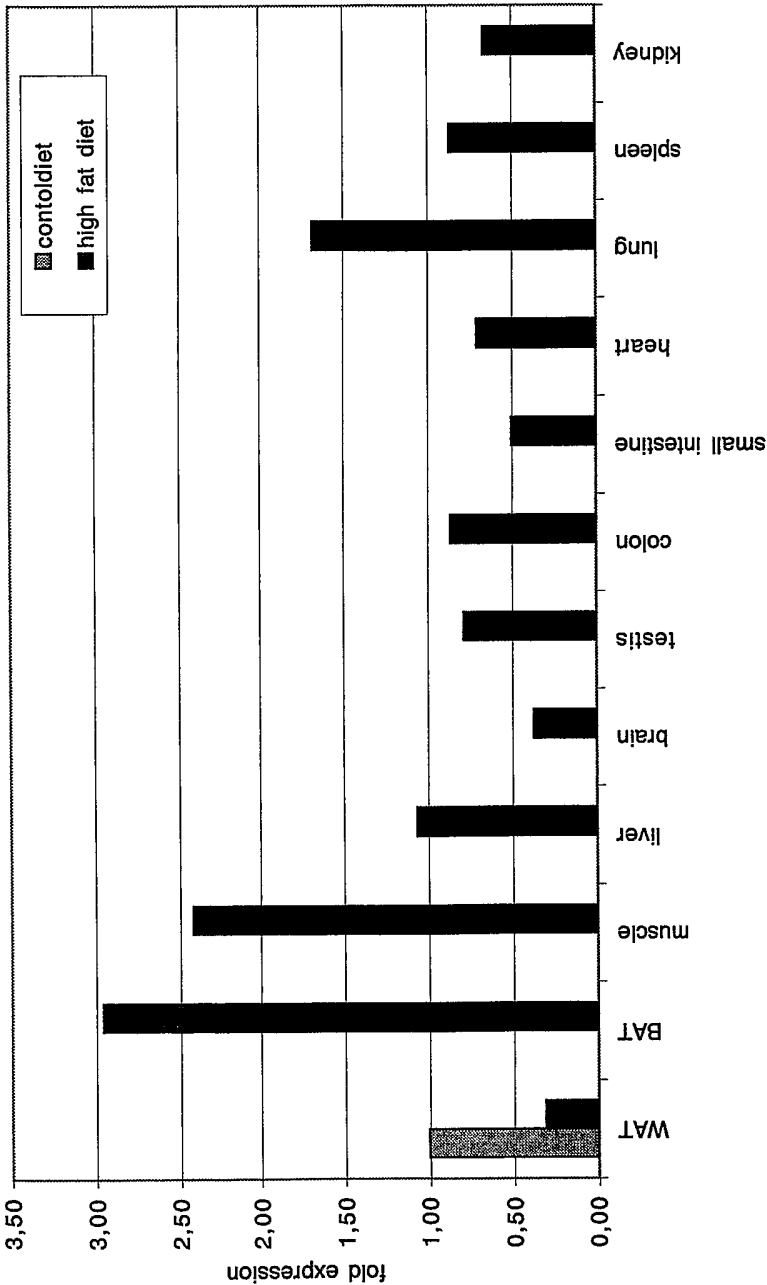


Figure 9D. Real-time PCR analysis of Slc7a1 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

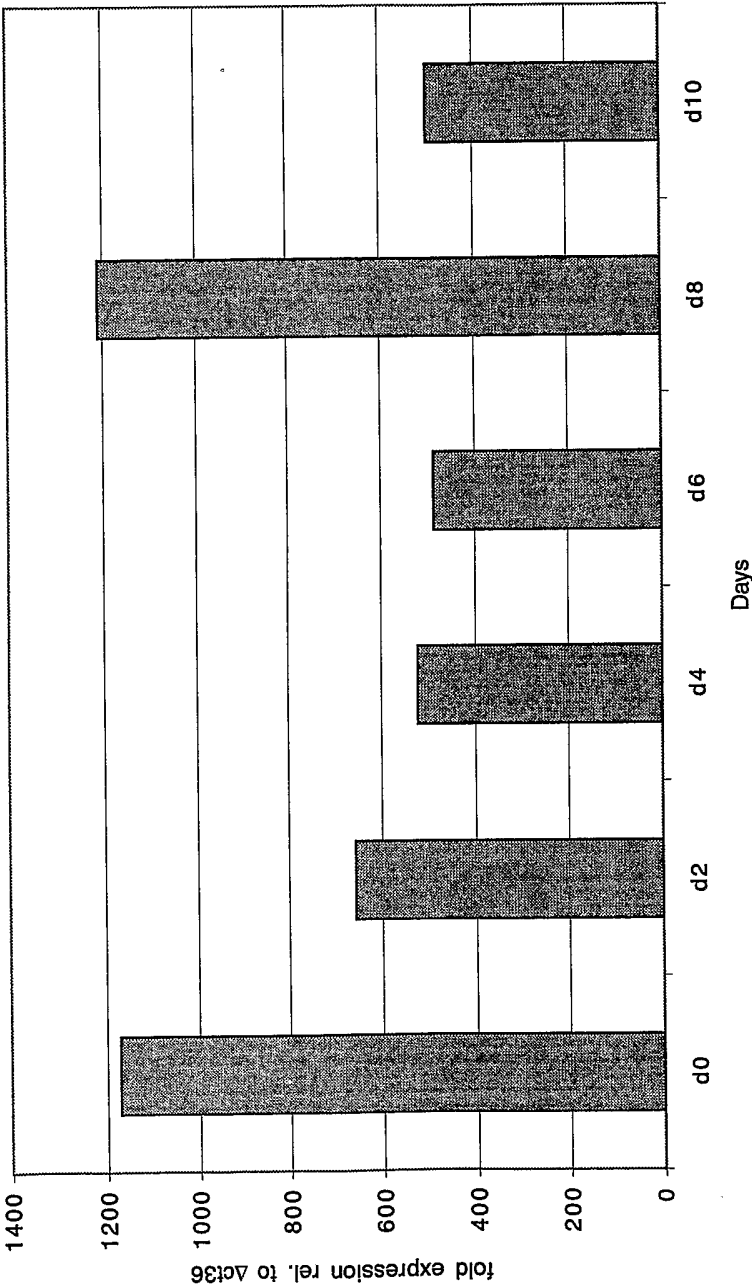


Figure 9E. Real-time PCR analysis of solute carrier family 7, member 2 (Slc7a2) expression in wild type mouse tissues

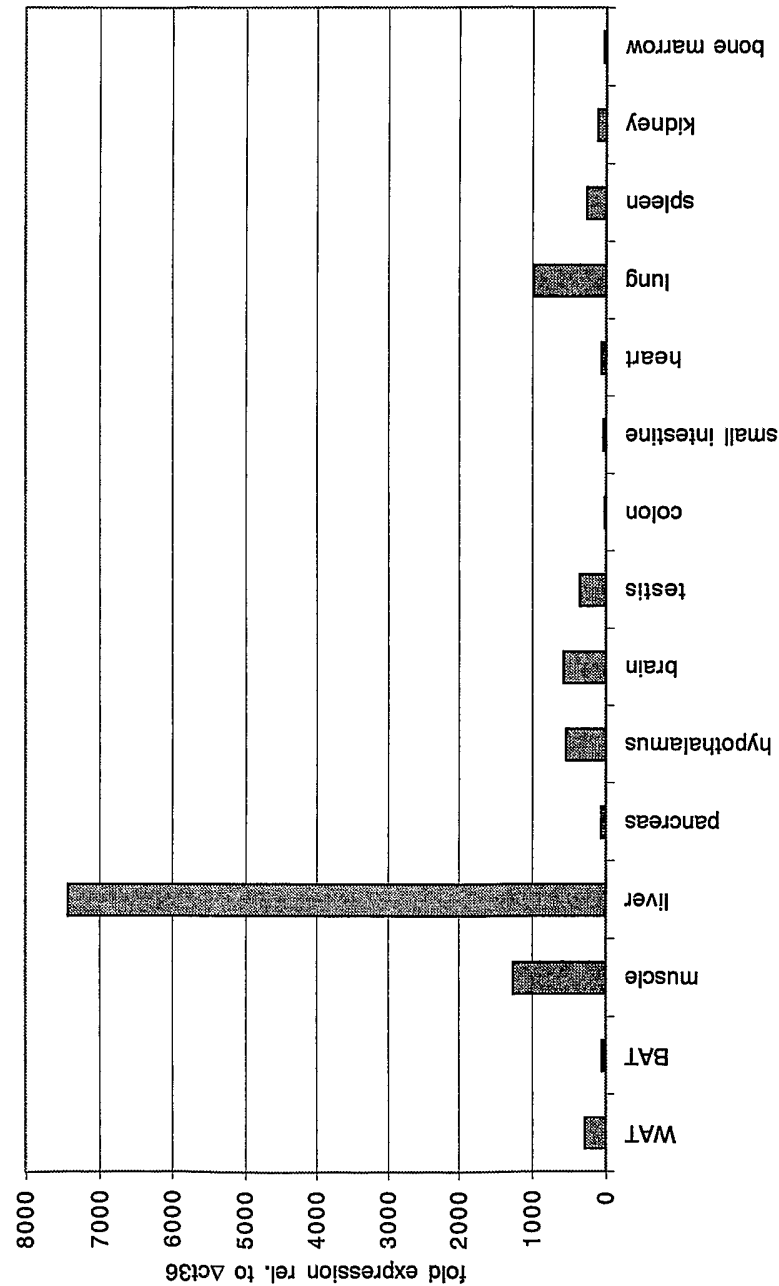


Figure 9F. Real-time PCR analysis of Slc7a2 expression in different mouse models

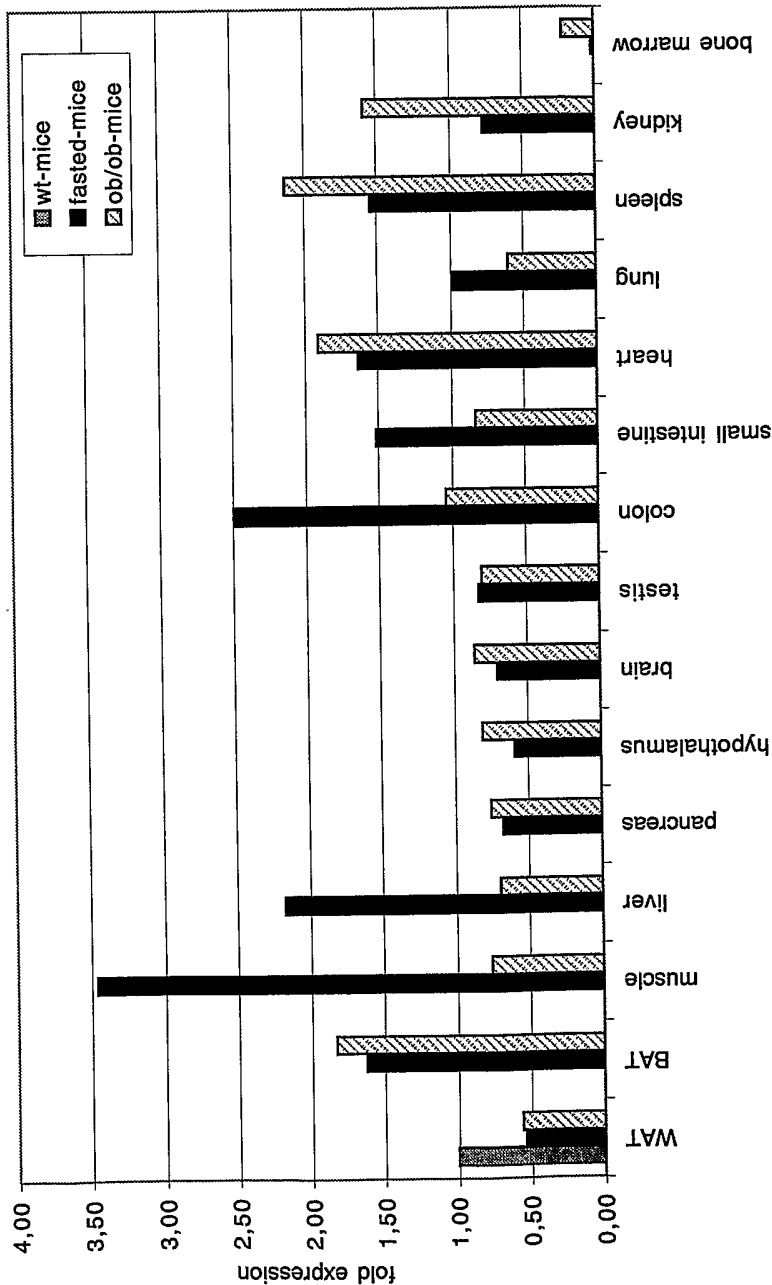


Figure 9G. Real-time PCR analysis of Slc7a2 expression in mice fed with a high fat diet compared to mice fed with a standard diet

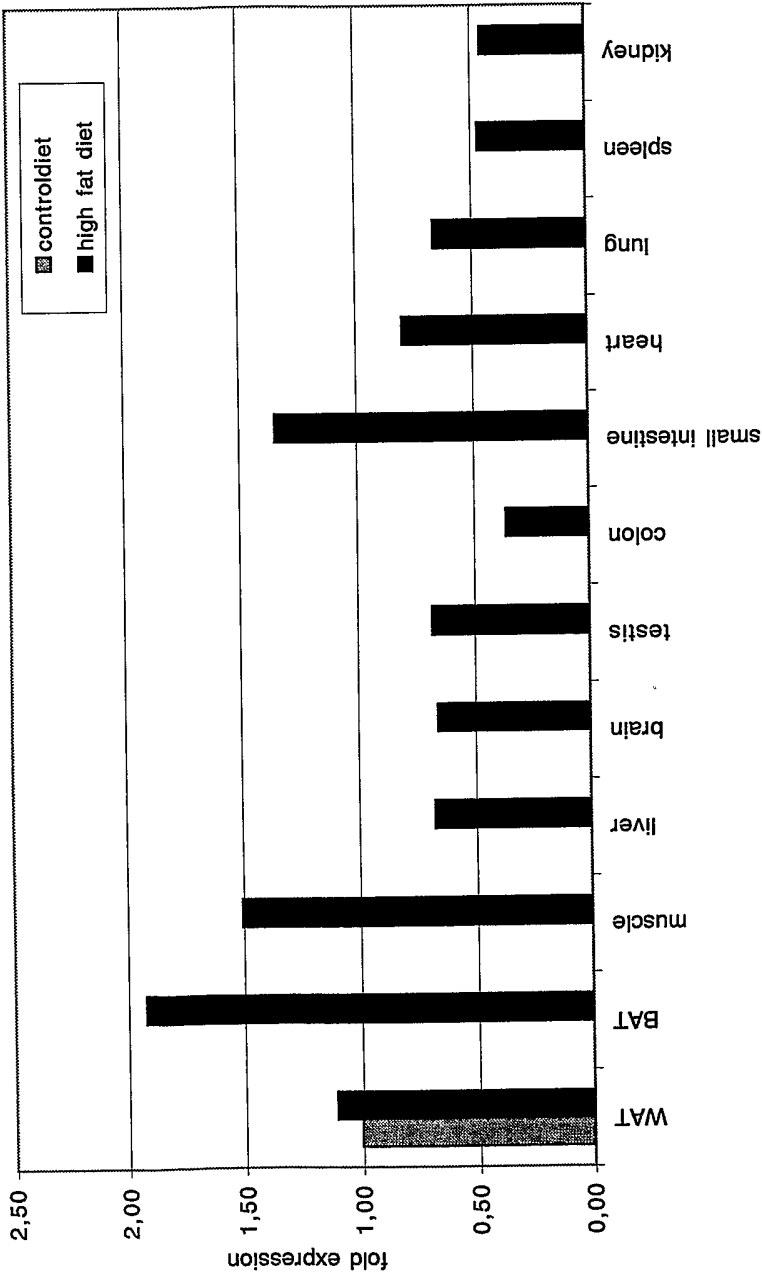


Figure 9H. Real-time PCR analysis of Slc7a2 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

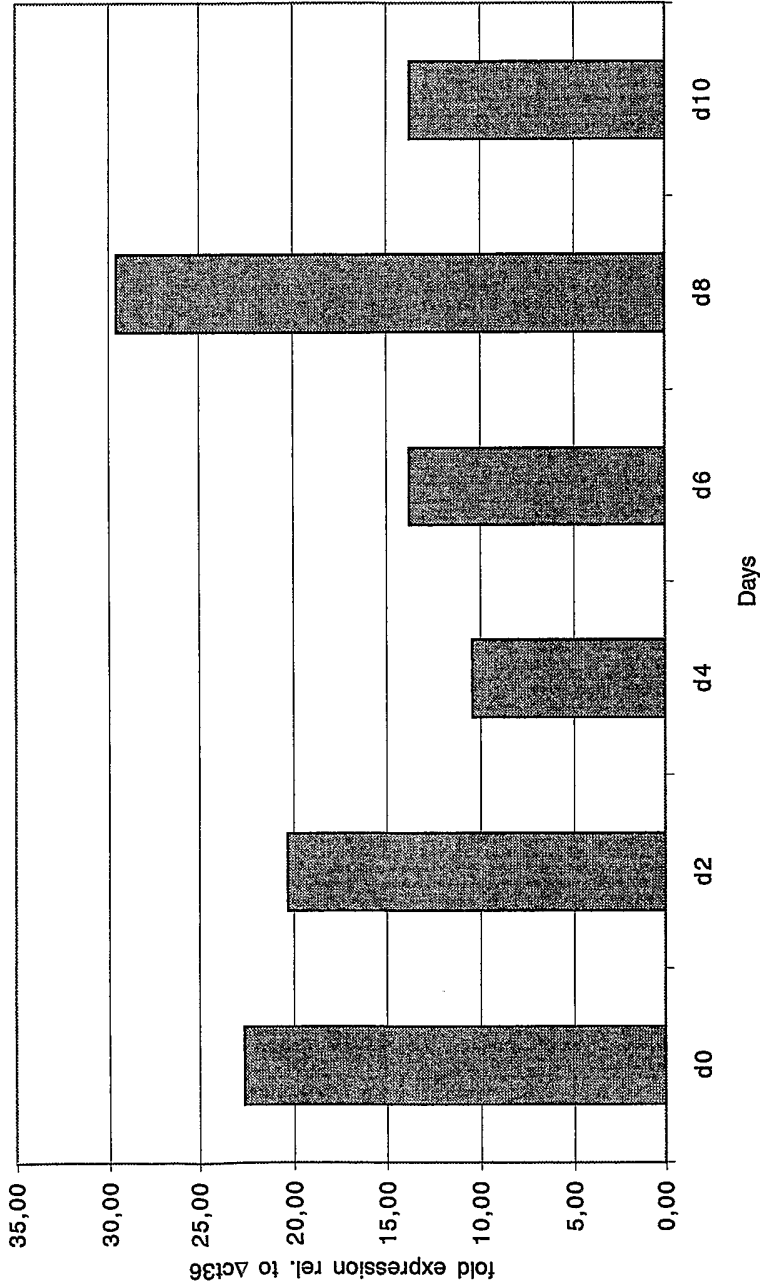


Figure 9L. Real-time PCR analysis of solute carrier family 7, member 3 (Slc7a3) expression in wild type mouse tissues

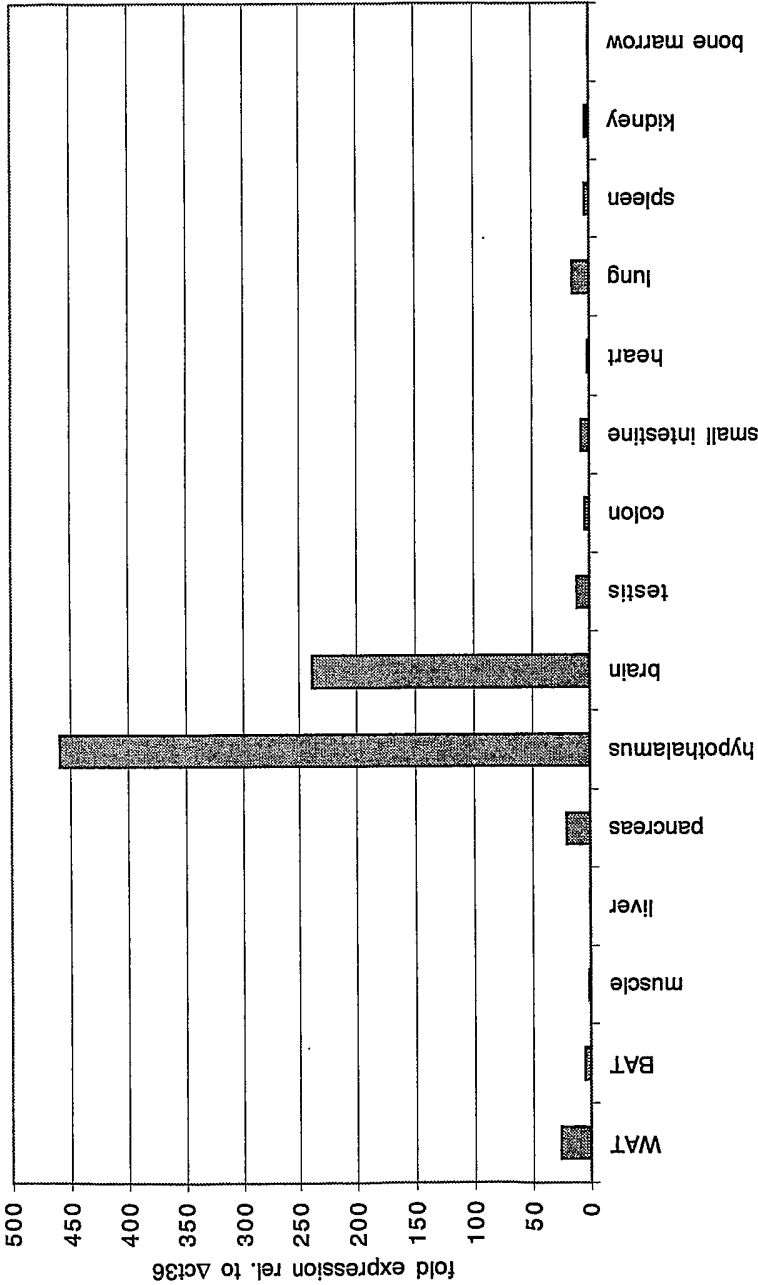


Figure 9J. Real-time PCR analysis of Slc7a3 expression in different mouse models

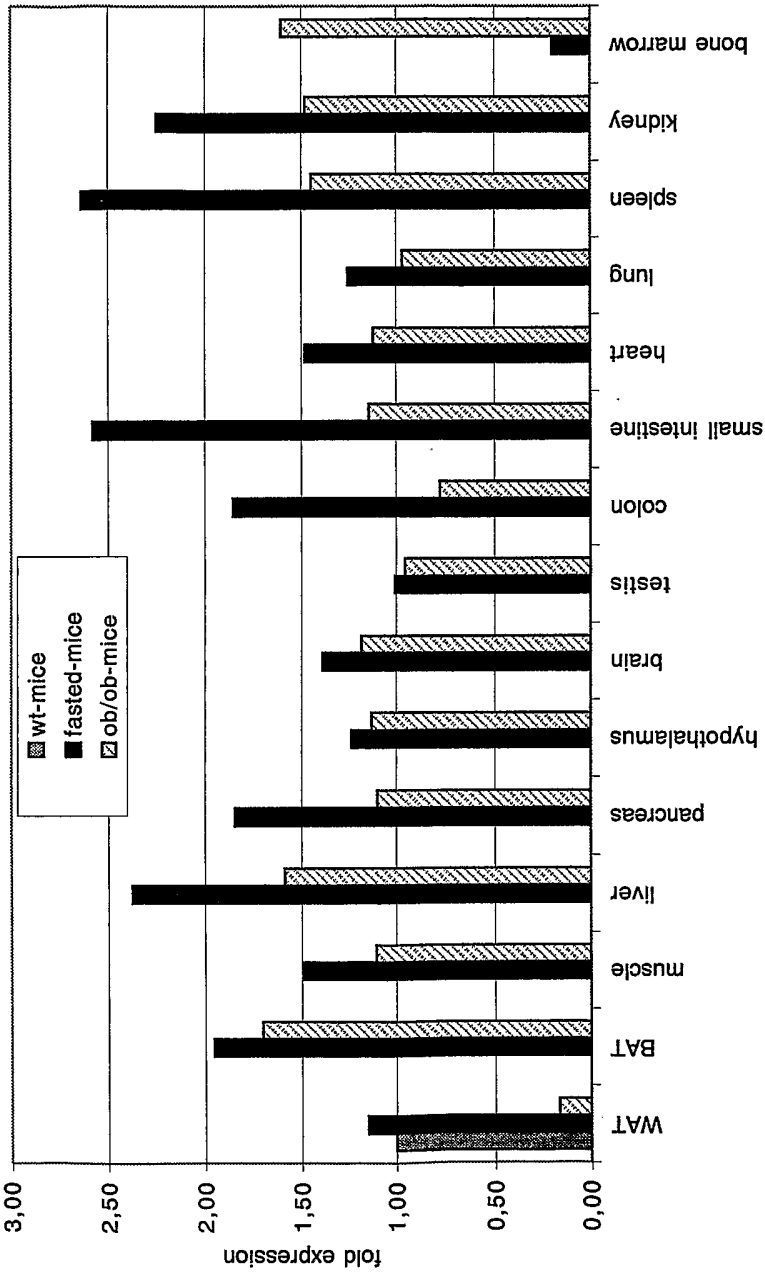


Figure 9K. Real-time PCR analysis of Slc7a3 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

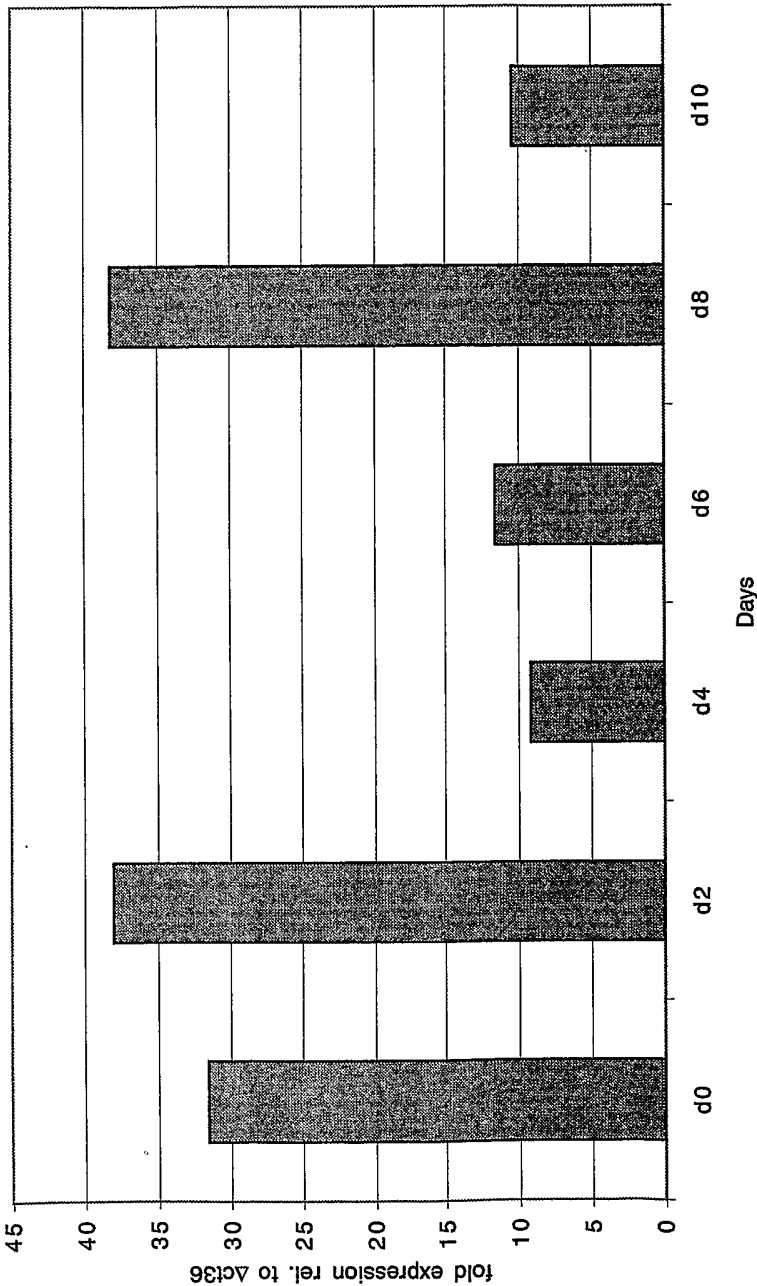


Figure 10. Triglyceride content of a *Drosophila* CG14217 (GadFly Accession Number) mutant

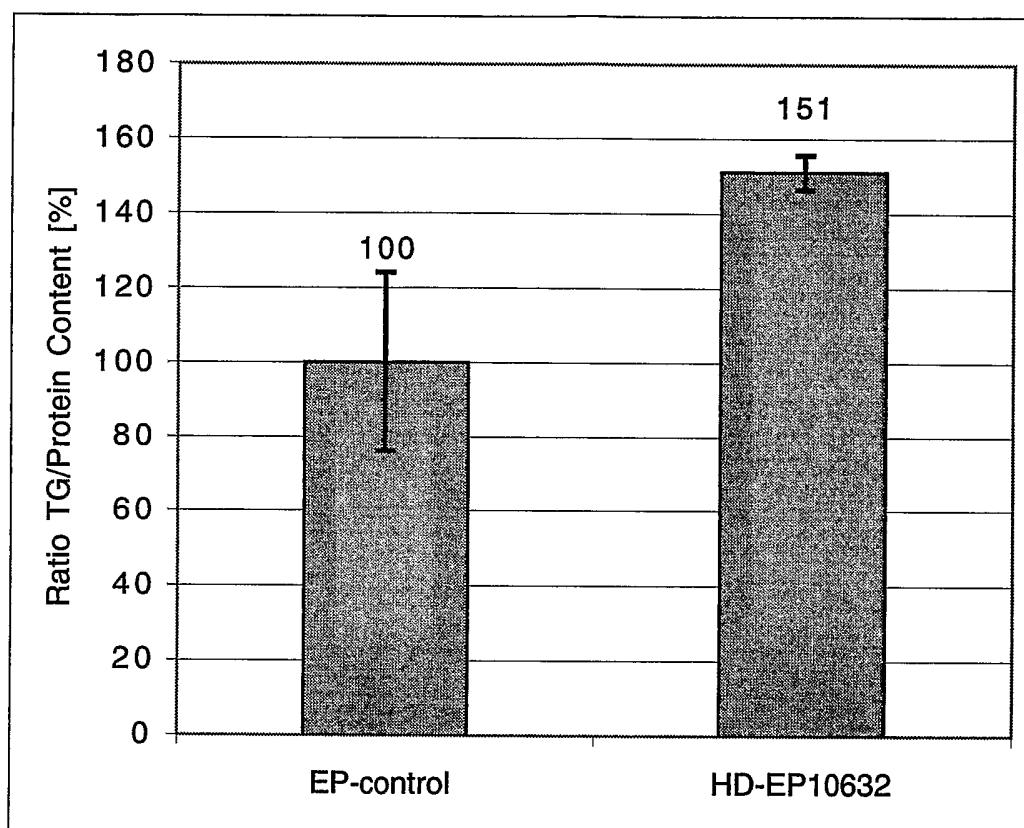


Figure 11. Molecular organization of the CG14217 gene (GadFly Accession Number)

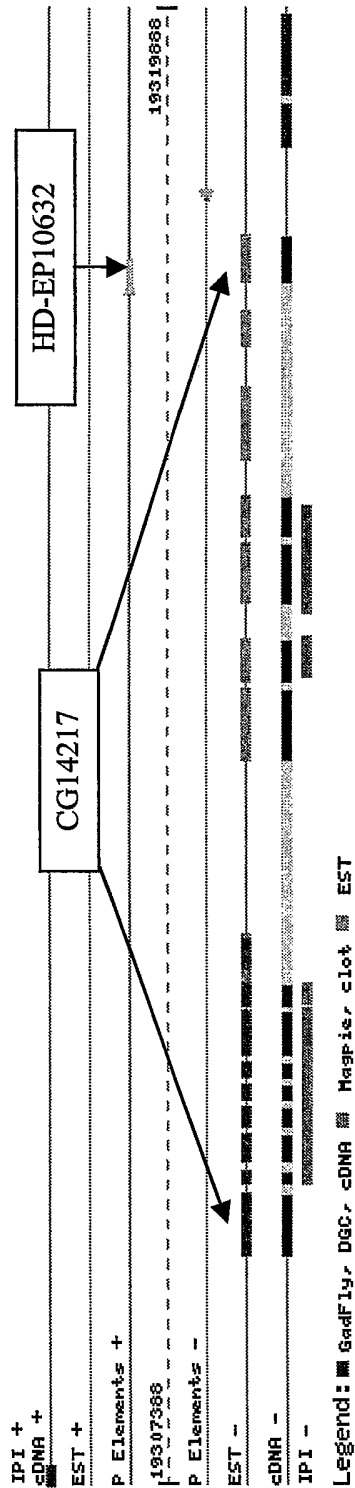


Figure 12. BLASTP results for CG14217 (GadFly Accession Number)**Homology to human protein NP_057235.1 (GenBank Accession Number)**

gi|7706401|ref|NP_057235.1| (NM_016151) prostate derived STE20-like kinase PSK [Homo sapiens], Length = 1235

Score = 464 bits (1193), Expect = e-130

Identities = 233/316 (73%), Positives = 268/316 (84%), Gaps = 1/316 (0%)

Query: 1 MPS-ARPGSLKDPETADLFNKHDPKIFEDLREIGHGSFGAVVYARCNLTREIVAICKMS 59
MP+ R GSLKDP++A+LF K DPEK+F DLREIGHGSFGAVY+AR E+VAICKMS
Sbjct: 1 MPAGGRAGSLKDPDVAELFFKDDPEKLFSDLREIGHGSFGAVYFARDVRNSEVVAICKMS 60

Query: 60 YTGKQSQEKWQDILKEIRFLRQLNHPNTIEYKGCYLRESTAWLVMEYCVGSASDIIEVHK 119
Y+GKQS EKWQDI+KE+RFL++L HPNTI+Y+GCYLRE TAWLVMEYC+GSASD++EVHK
Sbjct: 61 YSGKQSNEKWQDIIKEVRFLQKLRHPNTIQYRGCYLREHTAWLVMEYCLGSASDLLEVHK 120

Query: 120 KPLHEDEIAAICLGVLSGLSYLHSLGRIHRDIKAGNILLTDNGVVKLADFGSAAIKCPAN 179
KPL E EIAA+ G L GL+YLHS IHRD+KAGNILL++ G+VKL DFGSA+I PAN
Sbjct: 121 KPLQEVEIAAVTHGALQGLAYLHSHNMIHRDVKAGNILLSEPLVKLGDFGSASIMAPAN 180

Query: 180 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPPYFNMNAMSALYHIAQN 239
SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPP FNMNAMSALYHIAQN
Sbjct: 181 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPPFLNMNAMSALYHIAQN 240

Query: 240 ESPTLPKNDWSDAFCSFVELCLKKMPAERPSSAKLLTHAYVTRPRSDTVLLELIARTKSA 299
ESP L WS+ F +FV+ CL+K+P +RP+S LL H +V R R TV+++LI RTK A
Sbjct: 241 ESFVLQSGHWSEYFRNFVDSCLQKIPQDRPTSEVLLKHRFVLRERPPPTVIMDLIQRTKDA 300

Query: 300 VRELDNLNRYRKMKKIL 315
VRELDNL YRKMKKIL
Sbjct: 301 VRELDNLQYRKMKKIL 316

Score = 160 bits (406), Expect = 7e-39

Identities = 103/280 (36%), Positives = 160/280 (56%), Gaps = 6/280 (2%)

Query: 499 PASGGPLADRIQPVQPRYLTPAAQAAYVAASSASSQQAISNAVNDHGPNNFATIRTTSI 558
P S D QP P A A + +S++ ++A DH FATIRT S+
Sbjct: 420 PGSDNLYDDPYQPEITPSPLQPPAAPAPTSTTSSARRRAYCRN-RDH----FATIRTSAL 474

Query: 559 VTKQQQKEHMQEE-MHEQMSGYKMRREHQAHLVKLEEKCKVDMEAHKTALDKEYDTLLHN 617
V++Q +EH Q+ + EQ+SGYKMRMR+HQ L+ LE + + + E H L +E +
Sbjct: 475 VSRQIQEHEQDSALREQLSGYKMRMRHQKQLLALESRLRGEREHSARLQRELEAQRAG 534

Query: 618 FTRDLDRLETKHQQDVERRAKQTSAAEKKLHKEITLKQENDRKVYDLNRKKEYKANKERW 677
F + ++L +HQ E+ A+ A E+K + I +Q+ + +K+ YK KE+
Sbjct: 535 FGAEAEKLARRHQAIGEKEARAAQAERKFQQHILGQQKKELAALEAQKRTYKLRKEQL 594

Query: 678 KRELSMDESTPKRQRDLTLQSQKDNLKQHEAQEEQRMQLQAQKQYIELEMRKFKRKRMIQ 737
K EL + STPKR++ L QK+ L+Q +A+EE +L+ Q+QY EL+ R++KRK ++ +
Sbjct: 595 KEELQENPSTPKREKAEWLLRQKEQLQCCQAEAEAGLLRRQRQYFELQCROYKRKMILLAR 654

Query: 738 HEHEDQQLRDELGKKEQQLQQAAMLLKHHEKTQEELEYRQ 777
H + LR++L KK+ Q A+LL+ HE T+ELE RQ
Sbjct: 655 HSLDQDLLREDLNKKQTQKDLECALLLRQHEATRELELRQ 694

Score = 48.5 bits (114), Expect = 6e-05

Identities = 63/272 (23%), Positives = 118/272 (43%), Gaps = 41/272 (15%)

Query: 694 LTLQSQKDNLLKQHEAQEEQRMQLQAQKQYIELEMRKFKRKRMMIMQHEHEDQQLRDELGKKE 753
 L L+S+ ++ + QR L+AQ+ E K R+ +G+KE
 Sbjct: 507 LALESRLRGEREEHSARLQRELEAQRAGFGAEAEKLARR-----HQAIGEKE 553

Query: 754 QQLQQAHAAMLLKHH---EKTQELE--YRQKSVHQLREEQINKQHDTELHNQKDYMDRIK 808
 + QA + H ++ +EL QK ++LR+EQ+ + EL R K
 Sbjct: 554 ARAAQAEERKFQQHILGQKKELAALEAQKRTYKLRKEQLKE----ELQENPSTPKREK 609

Query: 809 KELVRKHAVELRQOPKSLKQKELQIRKQFRETCKTQTKQYKRYKAQVLQTPKEQQKEVI 868
 E + + +L+Q ++E + ++ R+ + Q +QYKR + ++ +E +
 Sbjct: 610 AEWLLRQKEQLQQ---CQAEEEAGLLRRQRYFELQCRQYKRMLLARHSLDQDLLREDL 666

Query: 869 KQLKEEKHRKLTLLGEQYEQSIADMFQSQSYKLDESQVIECQRTHEQLEYELEMILTAYQN 928
 + + +K + LL Q+E + +L+ Q+ QRT +L L+ T N
 Sbjct: 667 NKKQTQKDLECALLLRQHE-----ATRELELRQLQAVQRTAELT-RLQHQTTELGN 716

Query: 929 K---NKKQAEQQRDR---ERRELENRVSVRRG 954
 + NK++ QE R + + R+ + VR G
 Sbjct: 717 QLEYNKRREQELRQKHAAQVRQOPKSLKVRAG 748

Score = 46.2 bits (108), Expect = 3e-04

Identities = 65/282 (23%), Positives = 117/282 (41%), Gaps = 49/282 (17%)

Query: 733 RMIMQHEHEDQQLRDELGKKEQQLQQAHAAMLLKHHEKTQELEYRQKSVHQLREEQINKQ 792
 R I +HE +D LR++L ++ +Q LL E R ++ H R ++ +
 Sbjct: 477 RQIQEHE-QDSALREQLSGYKRMRRQHQQKQLL-----ALESRLRGEREEHSARLQRELEA 530

Query: 793 HDTELHNQKDYMDRIKKELVRKHAVELRQOPKSLKQKEL-QIRKQFRETCKTQTKQYKRY 851
 + + + R + + K A + + + +Q L Q +K+ + Q + YK
 Sbjct: 531 QRAGFGAEAEKLARRHQAIGEKEARAAQAEERKFQQHILGQKKELAALEAQKRTYKLR 590

Query: 852 KAQVLQTPKEQQKEVIKQLKEEKHRKLTLLGEQYEQSIADMFQSQSYKLDESQVIECQR 911
 K Q+ KE+ +E K EK L EQ +Q A+ +E+ ++ QR
 Sbjct: 591 KEQL-----KEELQENPSTPKREKAEWLLRQKEQLQQCQAE-----EEAGLLRRQR 636

Query: 912 THEQL---EYELEMLTA-----YQNKNNKQAEQ-----RDRERRELE 946
 + +L +Y+ +ML A ++ NKKQ Q+ R+ E R+L+
 Sbjct: 637 QYFELQCRQYKRMLLARHSLDQDLLREDLNKKQTQKDLECALLLRQHEATRELELRQLQ 696

Query: 947 NRVSVRGLENKMDAELQ---QFNQERAERLRMKHEKHTKE 985
 R L + EL ++N+ R + LR KH ++
 Sbjct: 697 AVQRTAELTRLQHQTTELGNQLEYNKRREQELRQKHAAQVRQ 738

Homology to human protein BAA92599.1 (GenBank Accession Number)

gi|7243103|dbj|BAA92599.1| (AB037782) KIAA1361 protein [Homo sapiens], Length = 1005

Score = 456 bits (1174), Expect = e-128

Identities = 271/437 (62%), Positives = 323/437 (73%), Gaps = 3/437 (0%)

Query: 1 MPSA-RPGSLKDPEIADLFNKHDPEKIFEDLREIGHGSFGAVYYARCNLTREIVAIIKMS 59
 MPS R GSLKDPEIA+LF K DPEK+F DLREIGHGSFGAVY+AR T E+VAIKKMS
 Sbjct: 5 MPSTNRAGSLKDPEIAELFFKEDPEKLFDTDLREIGHGSFGAVYFARDVRTNEVVAIKKMS 64

Query: 60 YTGKQSQEKWQDILKEIRFLRLNHPNTIEYKGCYLRESTAWLVMEYCVGSASDIIIEVHK 119
 Y+GKQS EKWQDI+KE++FL+++ HPN+IEYKGCYLRE TAWLVMEYC+GSASD++EVHK
 Sbjct: 65 YSGKQSTEKWQDIIKEVKFLQRIKHPNSIEYKGCYLREHTAWLVMEYCLGSASDLEEVHK 124

Query: 120 KPLHEDEIAAICLGVLISGLSYLHSLGRHRIKAGNILLTDNGVVKLADFGSAAIKCPAN 179
 KPL E EIAAI G L GL+YLHS IHRDIKAGNILLT+ G VKLADFGSA++ PAN
 Sbjct: 125 KPLQVEVEIAAITHGALQGLAYLHSHSTMIHRDIKAGNILLTEPGQVKLADFGSASMSPAN 184

Query: 180 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGITCIELAERKPPYFNMNAMSALYHIAQN 239
 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGITCIELAERKPP FNMNAMSALYHIAQN
 Sbjct: 185 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGITCIELAERKPPFLFNMNAMSALYHIAQN 244

Query: 240 ESPTLPKNDWSDAFCSFVELCLKKMPAERPSSAKLLTHAYVTRPRSDTVLLELIARTKSA 299
 ESPTL N+WSD F +FV+ CL+K+P +RP+S +LL H +V R R +TVL++LI RTK A
 Sbjct: 245 ESPTLQSNWSDYFRNFVDSCLQKIPQDRPTSEELLKHIFVLRERPETVLIDLIQRTKDA 304

Query: 300 VRELDNLNRYKMKKILMVDTCETESAVGDTDDQDDHAGGDSSKSNITSEHSIHVGVVS 359
 VRELDNL YRKMKK+L + + + + + DH G + NS+ S SI
 Sbjct: 305 VRELDNLQYRKMKKLLFQEAHNGPAVEAQEEEEQDHGVGRTGTVNSVGSNQSI-PSMSI 363

Query: 360 AASSQSSSSNSIPAAAQNHHAHAAHHHQQAASAAVAAMHHHHHPHQPPPSWPSGQQGQ 419
 +ASSQSSS NS+P + + + + + + ++ +H P +
 Sbjct: 364 SASSQSSSVNSLPDVSDDKSELDMMEGDHTVMSN-SSVIHLKPEEENYREEGDPRTASD 422

Query: 420 PVPPGAVSRNSSRHRNR 436
 P P VSR+ S +RNR
 Sbjct: 423 PQSPQVSRHKSHYRNR 439

Score = 401 bits (1031), Expect = e-111

Identities = 232/494 (46%), Positives = 335/494 (66%), Gaps = 1/494 (0%)

Query: 528 AASSASSQQAISNAVNDHGPNNFATIRTTTSIVTKQQKEHMQE-EMHEQMSGYKRMRRHQ 586
 A+ S Q + + +FATIRT S+VT+Q +EH Q+ E+ EQMSGYKRMRR+HQ
 Sbjct: 420 ASDPQSPQVSRHKSHYRNRHFATIRTTASLVTRQMQEHEQDSELREQMSGYKRMRRHQ 479

Query: 587 AHLVKLEEKCKVDMEAHKTALDKEYDTLLHNFTRDLDRLETKHQQDVERRAKQTSAAEKK 646
 L+ LE K K +M+ H+ LDK+ +T +NF ++++L KHQ +E+ AK S EKK
 Sbjct: 480 KQLMTLENKLKAEMDEHRLRLDKDLETQRNNFAAEMEKLKHKQAAMEKEAKVMSNEEKK 539

Query: 647 LHKEITLKQENDRKVYDLNRKKEYKANKERWKRELSMDESTPKRQRDLTLQSQKDNLKQH 706
 + I +Q+ + + ++K+EYK KE+ K EL+ ++STPK+++ L QK+N++
 Sbjct: 540 FQHQHQAQQKELNSFLESQKREYKLRKEQLKEELNENQSTPKKEKQEWLSKQKENIQHF 599

Query: 707 EAQEEQRMQLQAQKQYIELEMRKFKRKRIMQHEHEDQQLRDELGKKEQQLQAAMLLKH 766
 +A+EE +L+ Q+QY+ELE R+FKR+ ++ +H E +R+EL K++ Q HAML+
 Sbjct: 600 QAEEEAANLRRQRQYLELECRRFKRRLGLRHNLQDLVREELNKRQTQKDLHAMLRLQ 659

Query: 767 HEKTQELEFRQKSVHQLREEQINKQHDTELHNQKDYMDRIKKELVRKHAVELRQQPKSL 826
 HE QELE+R ++ ++R E I QH TEL NQ +Y R ++EL RKH +E+RQQPKSL
 Sbjct: 660 HESMQELEFRHLNTIQKMRCELIRLQHQTETLNQLEYNKRERELRRKHVMEVRQQPKSL 719

Query: 827 KQKELQIRKQFRETCKTQTKQYKRYKAQVLQTPKEQQKEVIKQLKEEKHRKLTLGGEQY 886
 K KELQI+KQF++TCK QT+QYK + +L+TPK + K V+K+LKEE+ RKL +L EQY
 Sbjct: 720 KSKELQIKKQFQDTCKIQTRQYKALRNHLLTTPKSEHKAVLKRLEEQTRKLAILAEQY 779

Query: 887 EQSIADMFQSQSYKLDESQVIECQRTHEQLEVELEMLTAYQNKNKKQAQEQDRERRELE 946
 + SI +M +Q+ +LDE+Q ECQ QL+ ELE+L AYQ+K K QA+ Q DRE RELE
 Sbjct: 780 DHSINEMLSQALRLDEAQEAECQVLKMLQQLQLELLNAYQSKIKMQAEAQHDRELELE 839

Query: 947 NRVSVRRGLEENKMDAELQQFNQERAERLRMKHEKHTKELEAFDNESIALGFSTLSLIEV 1006
 RVS+RR LLE K++ E+ ER ER+R E+ +E+EAFD+ES+ LGFS + L +
 Sbjct: 840 QRVSLRRALLEQKIEEMLALQNERTERIRSLERQAREIEAFDSSEMRGLGFSNMVLSNL 899

Query: 1007 SREAYADEEGSLSG 1020
 S EA++ SG
 Sbjct: 900 SPEAFSHSYPGASG 913

Homology to human protein NP_057365.2 (GenBank Accession Number)

gi|19923464|ref|NP_057365.2| (NM_016281) STE20-like kinase; STE2-like kinase [Homo sapiens], Length = 898

Score = 440 bits (1132), Expect = e-123

Identities = 263/439 (59%), Positives = 316/439 (71%), Gaps = 15/439 (3%)

Query: 5 RPSGLKDPEIADLFNKHDPKIFEDLREIGHGSFGAVYYARCNTREIVAIAKKMSYTGKQ 64
 R G LKDPEIADLF K DPE++F L EIGHGSFGAVY+A T E+VAIKKMSY+GKQ
 Sbjct: 2 RKGVLKDPEIADLFYKDDPEELFIGLHEIGHGSFGAVYFATNAHTNEVVAIKKMSYSGKQ 61

Query: 65 SQEKWQDILKEIRFLRQLNHPNTIEYKGCYLRESTAWLVMEYCVGSASDIIEVHKKPLHE 124
 + EKWQDILKE++FLRQL HPNTIEYKGCYL+E TAWLVMEYC+GSASD++EVHKKPL E
 Sbjct: 62 THEKWQDILKEVKFLRQLKHPNTIEYKGCYLKEHTAWLVMEYCLGSASDLLEVHKKPLQE 121

Query: 125 DEIAAICLGVL SGLSYLHSLGRIHRDIKAGNILLTDNGVVKLADFGSAAIKCPANSFVGT 184
 EIAAI G L GL+YLHS IHRDIKAGNILLT+ G VKLADFGSA++ PANSFVGT
 Sbjct: 122 VEIAAITHGALHGLAYLHSHALHRDIKAGNILLTEPGQVKLADFGSASMASPANSFVGT 181

Query: 185 PYWMAPEVILAMDEGQYDGKVDVWSLGITCIELAERKPPYFNMNAMSALYHIAQNESPTL 244
 PYWMAPEVILAMDEGQYDGKVD+WSLGITCIELAERKPP FNMNAMSALYHIAQN+SPTL
 Sbjct: 182 PYWMAPEVILAMDEGQYDGKVDIWSLGITCIELAERKPPPLFNMNAMSALYHIAQNDSPTL 241

Query: 245 PKNDWSDAFCSFVELCLKKMPAERPSSAKLLTHAYVTRPRSDTVLLELIARTKSARELD 304
 N+W+D+F FV+ CL+K+P ERP+SA+LL H +V R R VL++LI RTK AVRELD
 Sbjct: 242 QSNEWTDSFRFVDYCLQKIPQERPTSAELLRHDFVRRDRPLRLVLDLIQRTKDAVRELD 301

Query: 305 NLNYRKMKIILMVDTCETESAVGDTDDQDDHAGGDSKSNSTSEHSIHSVGVSAASSQ 364
 NL YRKMKIL +T D++ +H + + +S+ S HSI S+ VS S
 Sbjct: 302 NLQYRKMKILFQETRNGPLNESQDEEDSEHGTSLNREMDSLGNSHSIPMSVSTGSQS 361

Query: 365 SSSSNSIPAAQNNHHHIAAHHHQAASA AVAAAMHHHHH-----PHQQPPPSWPSGQQ 417
 SS ++ ++ + H ++ + ++ +H H H P P
 Sbjct: 362 SSVNSMQEVMDESSSELVMMHDESTINSSSVVHKKDHVFIRDEAGHGDRP----- 414

Query: 418 GQPVPPGAVSRNSSRHRNR 436
 +P P +V + +RNR
 Sbjct: 415 -EPRPTQSVQSQALHYRNR 432

Score = 382 bits (980), Expect = e-105

Identities = 220/479 (45%), Positives = 318/479 (65%), Gaps = 1/479 (0%)

Query: 534 SQQAISNAVNDHGPNNFATIRTSIVTKQKQEHMQE-EMHEQMSGYKRMRRHQHVLKL 592
 +Q S A++ FATI++ S+VT+Q EH QE E+ EQMSGYKRMRR+HQ L+ L
 Sbjct: 419 TQSVQSQALHYRNRERFATIKSASLVTRQIHEHEQENELREQMSGYKRMRRHQHQLIAL 478

Query: 593 EEKCKVDMEAHKTALDKEYDTLLHNFTRLDRLETKHQQDVERRAKQTSAAEKKLHKEIT 652
 E K K +M+ H+ L KE +T +N + +L++L K +E+ AK +A EKK ++I
 Sbjct: 479 ENKLKAEMDEHRLKLQKEVETHANNSSIELEKLAKKQVAIEKEAKVAAADEKKFQQQIL 538

Query: 653 LKQENDRKVYDLNRKKEYKANKERWKRELSMDESTPKRQRDLTLQSQKDNLKQHEAQEEQ 712
 +Q+ D + ++KK+YK KE+ K E++ D STPK+++ + K+NL+ +A+EE
 Sbjct: 539 AQQKDLTTFLESQKQYKICKKEIKKEEMNEDHSTPKKEQERISKHENLQHTQAEAEA 598

Query: 713 RMLQAQKQYIELEMRKFKRKRMTMQHEHEDQQLRDELGKKEQQLQQAAMLLKHHEKTQE 772
 +L Q+ Y + R FKRK MI +HE E Q +R+EL KK Q + HAML++H E T+E
 Sbjct: 599 HLLTQQLRYDDKNCRFKRKIMIKRHEVEQQNIREELNKKRTQKEMEAMLI RHDESTRE 658

Query: 773 LEYRQOKSVHQLREEQINKQHDTELHNQKDYMDRIKKELVRKHAVELRQOPKSLKQKELQ 832
 LEYRQ ++ +LR + I QH TEL NQ +Y R ++EL RKH +ELRQOPK+LK E+Q
 Sbjct: 659 LEYRQLHTLQKLRLMDLIRLQHQTELENQLEYNKRRELERHKKHVMELRQOPKLNKAMEMQ 718

Query: 833 IRKQFRETCKTQTKQYKRYKAQVLQTPKEQQKEVIKQLKEEKHRKLTLLGEQYEQSIAD 892
 I+KQF++TCK QTKQYK K L+ TPK + K ++K LK+E+ RKL +L EQYEQSI +
 Sbjct: 719 IKKQFQDTCKVQTKQYKALKNHQLEVTPKNEHKTILKTLKDEQTRKLAILAEQYEQSINE 778

Query: 893 MFQSQSYKLDESQVIECQRTHEQLEYELEM/LTAYQNKNNKQAQEQDRDRERRELENRVSVR 952
 M SQ+ +LDE+Q ECQ QL+ E+E+L AYQ+K K Q + Q +RE ++LE RVS+R
 Sbjct: 779 MMASQALRLDEAQAECQALRLQLQEMELLNAYQSKIKMQTEAQAHERELQKLEQRVSLR 838

Query: 953 RGLLENKMDAELQQFNQERAERLRMKHEKHTKELEAFDNESIALGFSTLSLIEVSREAY 1011
 R LE K++ EL +ER+ER++ E+ +E+E FD ES+ +GF L ++ +E Y
 Sbjct: 839 RAHLEQKIEEELAALQKERSERIKNLLERQEREIETFDMESLRMGFGNLVTLDFPKEDY 897

Homology to human protein NP_004774.1 (GenBank Accession Number)

gi|4759208|ref|NP_004774.1| (NM_004783) thousand and one amino acid protein kinase
 [Homo sapiens], Length = 1049

Score = 439 bits (1128), Expect = e-122

Identities = 233/316 (73%), Positives = 268/316 (84%), Gaps = 1/316 (0%)

Query: 1 MPS-ARPGSLKDPEIADLFNKHDEPKIFEDLREIGHGSFGAVYYARCNLTREIVAIAIKMS 59
 MP+ R GSLKDP++A+LF K DPEK+F DLREIGHGSFGAVY+AR E+VAIAIKMS
 Sbjct: 1 MPAGGRAGSLKDPDVAELFFKDDPEKLFSDLREIGHGSFGAVYFARDVRNSEVVAIAIKMS 60

Query: 60 YTGKQSQEKWQDILKEIRFLRQLNHPNTIEYKGCYLRESTAWLVMEYCVGSASDIIEVHK 119
 Y+GKQS EKWQDI+KE+RFL++L HPNTI+Y+GCVLRE TAWLVMEYC+GSASD++EVHK
 Sbjct: 61 YSGKQSNEKWQDIIKEVRFLQKL RHPNTIQYRGCVLREHTAWLVMEYCLGSASDLLEVHK 120

Query: 120 KPLHEDEIAAICLGVL SGLSYLHSLGRIHRDIKAGNILLTDNGVVKLADFGSAAIKCPAN 179
 KPL E EIAA+ G L GL+YLHS IHRD+KAGNILL++ G+VKL DFGSA+I PAN
 Sbjct: 121 KPLQEVEIAAVTHGALQGLAYLHSHNMIHRDVKAGNILLSEPLVKLGDFGSASIMAPAN 180

Query: 180 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPPYFNMNAMSALYHIAQN 239
 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPP FNMNAMSALYHIAQN
 Sbjct: 181 SFVGTPTYWMAPEVILAMDEGQYDGKVDVWSLGTICIELAERKPPLFNMNAMSALYHIAQN 240

Query: 240 ESPTLPKNDWSDAFCSFVELCLKMPAERPSSAKLLTHAYVTRPRSDTVLLELIARTKSA 299
 ESP L WS+ F +FV+ CL+K+P +RP+S LL H +V R R TV+++LI RTK A
 Sbjct: 241 ESPVLQSGHWSEYFRNFVDSCLQKIPQDRPTSEVLLKHRFVLRERPPFTVIMDLIQRTKDA 300

Query: 300 VRELDNLNRYRKMCKIL 315
 VRELDNL YRKMCKIL
 Sbjct: 301 VRELDNLQYRKMCKIL 316

Figure 13. Expression of CG14217 Homologs in Mammalian Tissues
Figure 13A. Real-time PCR analysis of mouse RIKEN cDNA 1110033K02 gene (1110033K02Rik) expression in wild type mouse tissues

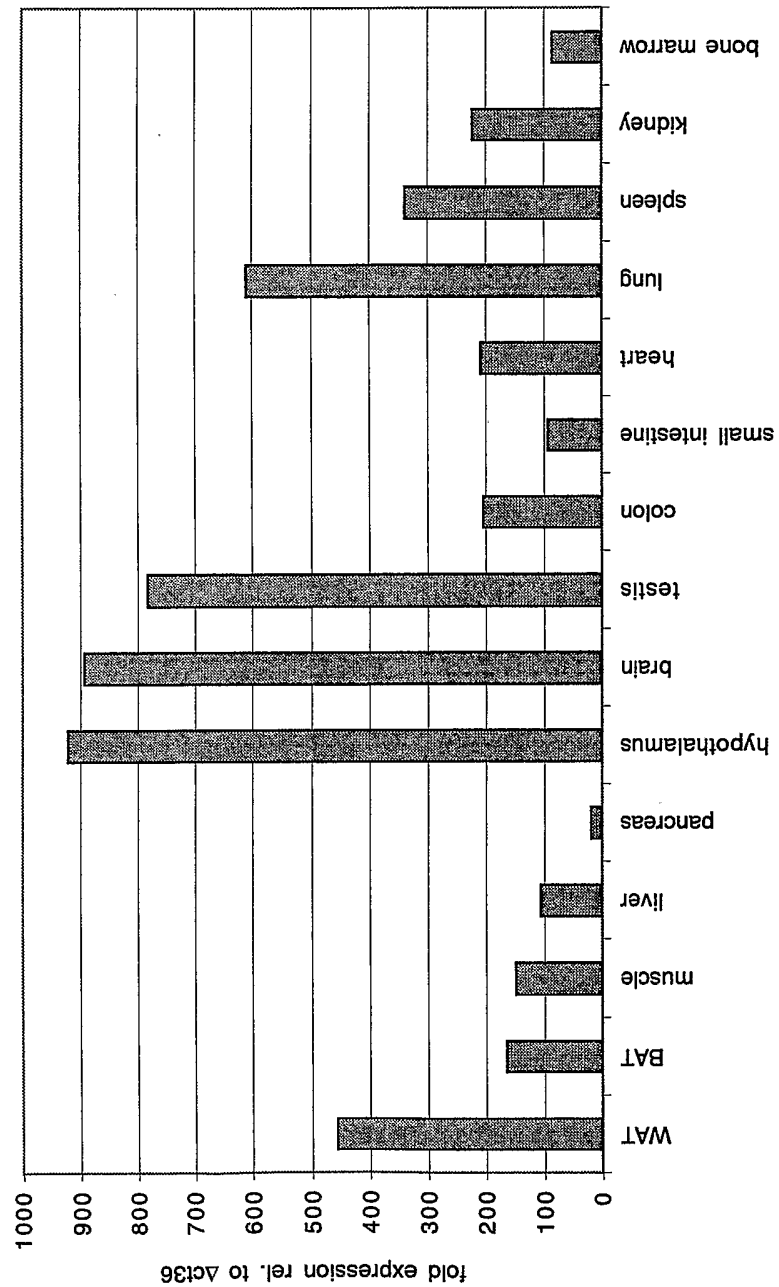


Figure 13B. Real-time PCR analysis of 1110033K02Rik expression in different mouse models

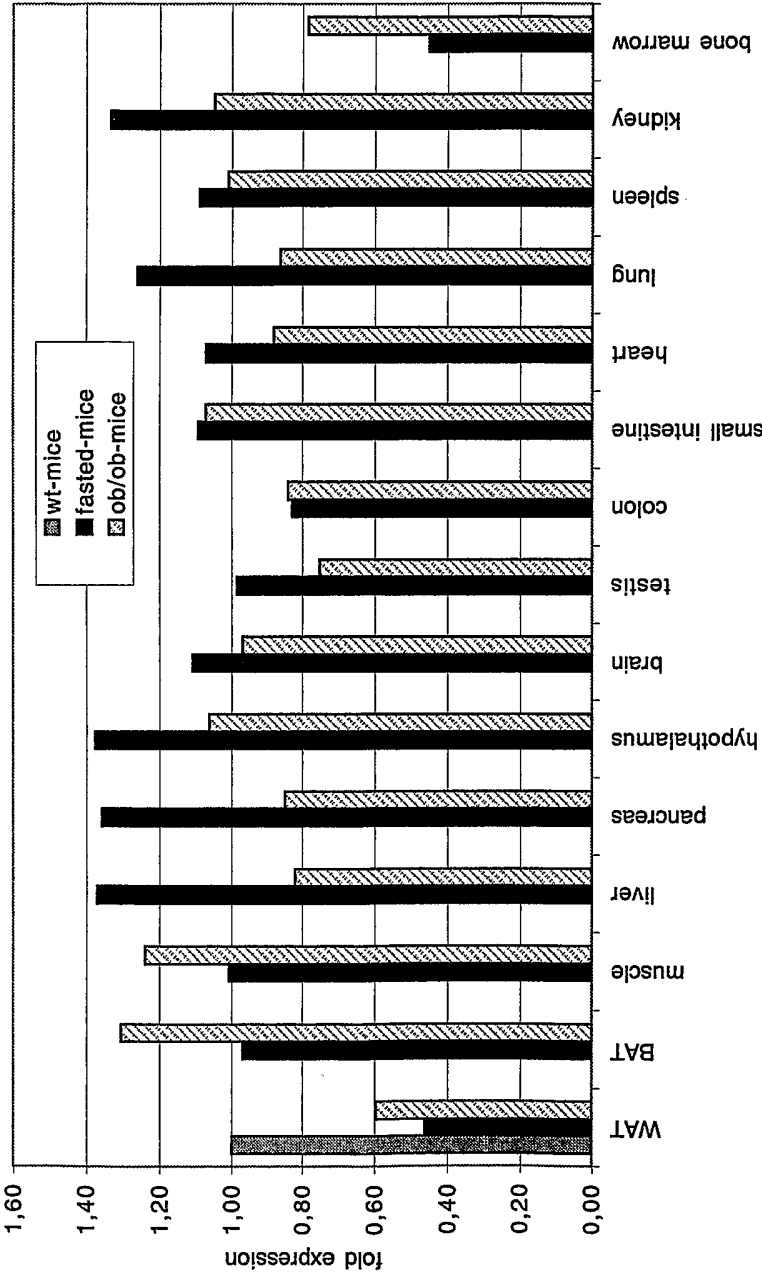


Figure 13C. Real-time PCR analysis of 1110033K02Rik expression in mice fed with a high fat diet compared to mice fed with a standard diet

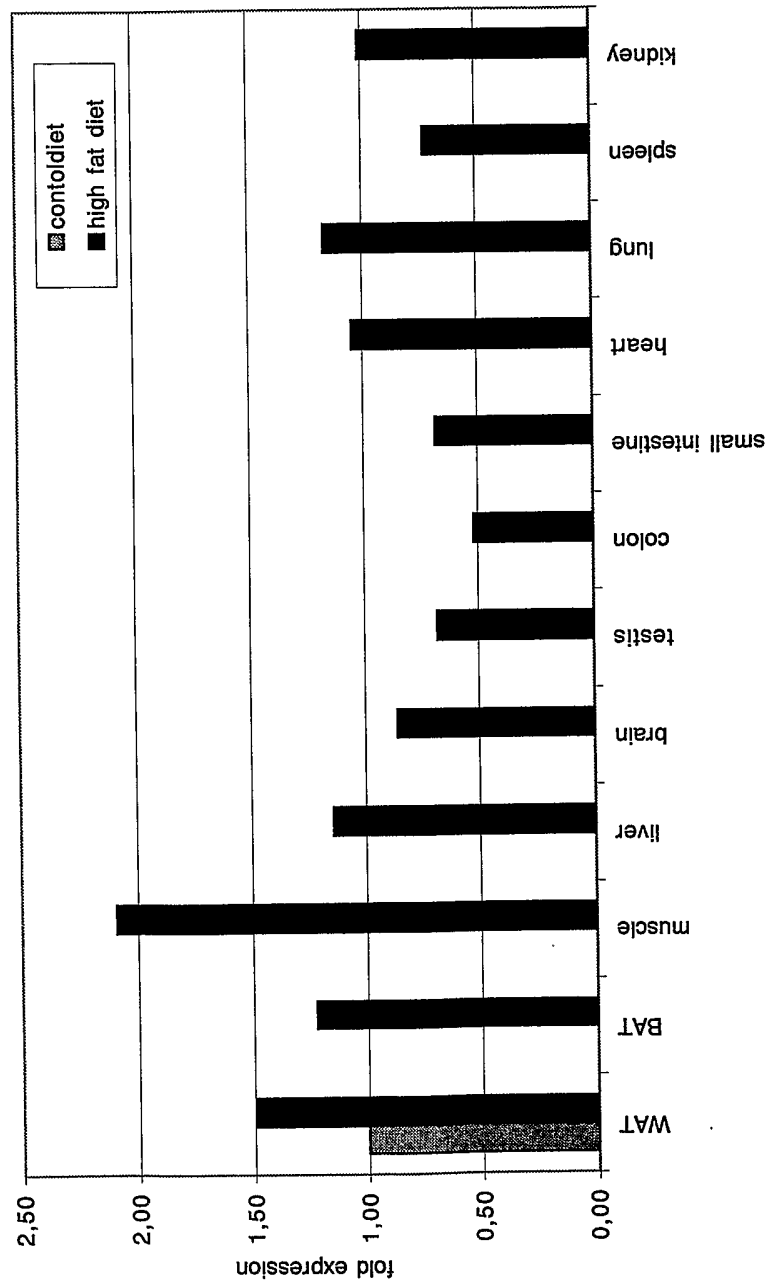


Figure 13D. Real-time PCR analysis of 1110033K02Rik expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

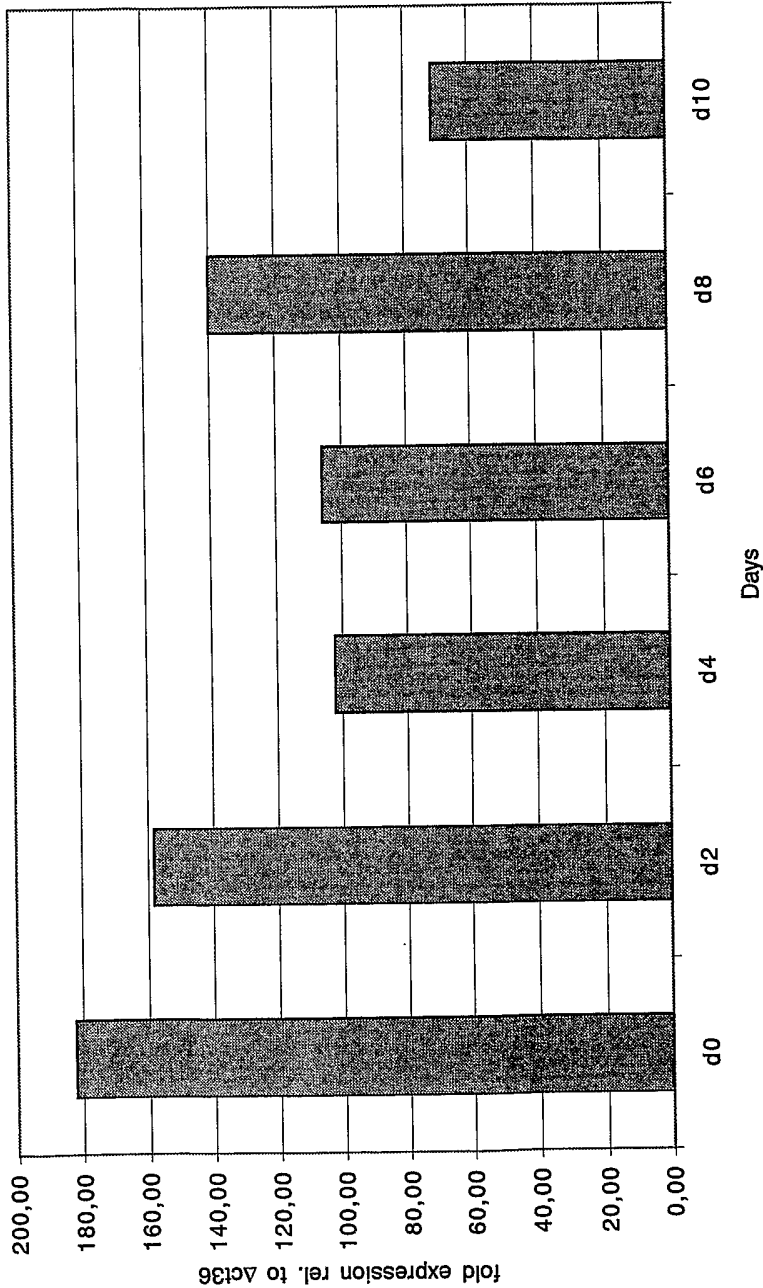


Figure 13E. Real-time PCR analysis of mouse protein similar to thousand and one amino acid protein kinase (LOC192767) expression in wild type mouse tissues

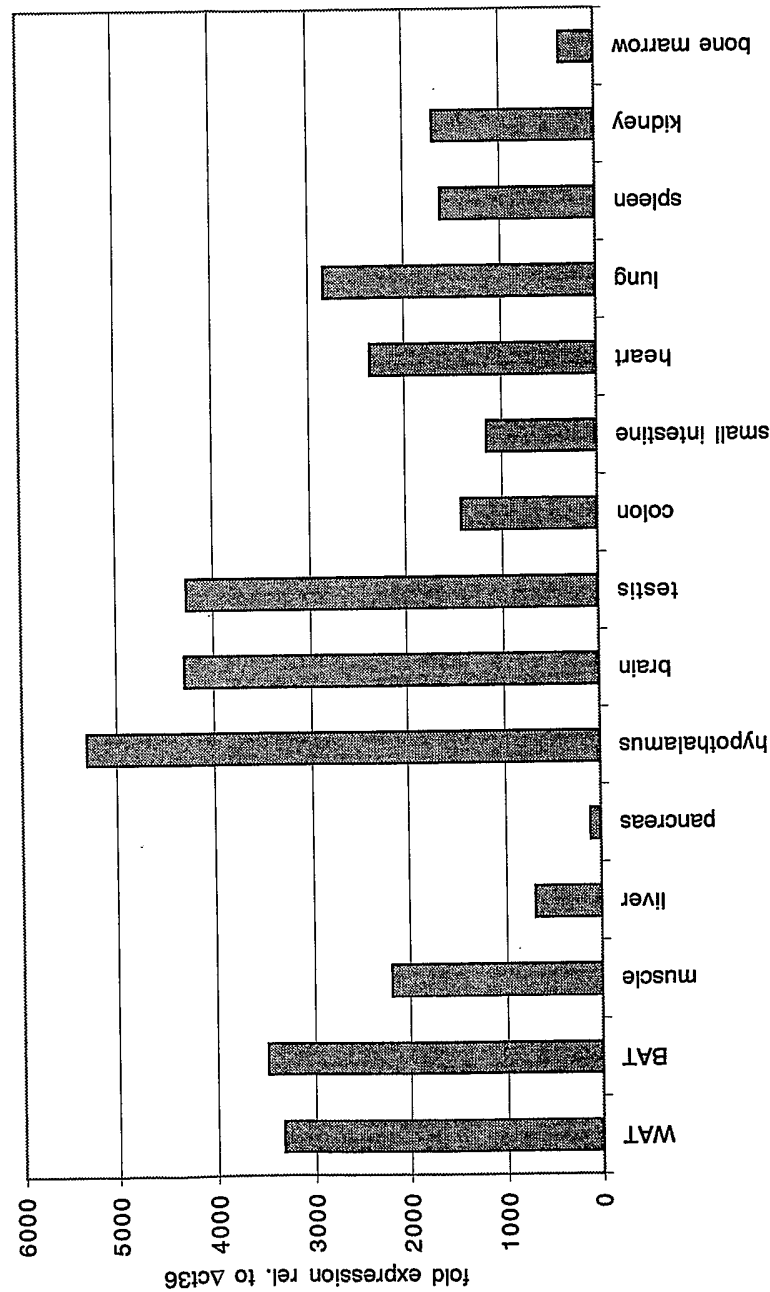


Figure 13F. Real-time PCR analysis of LOC192767 expression in different mouse models

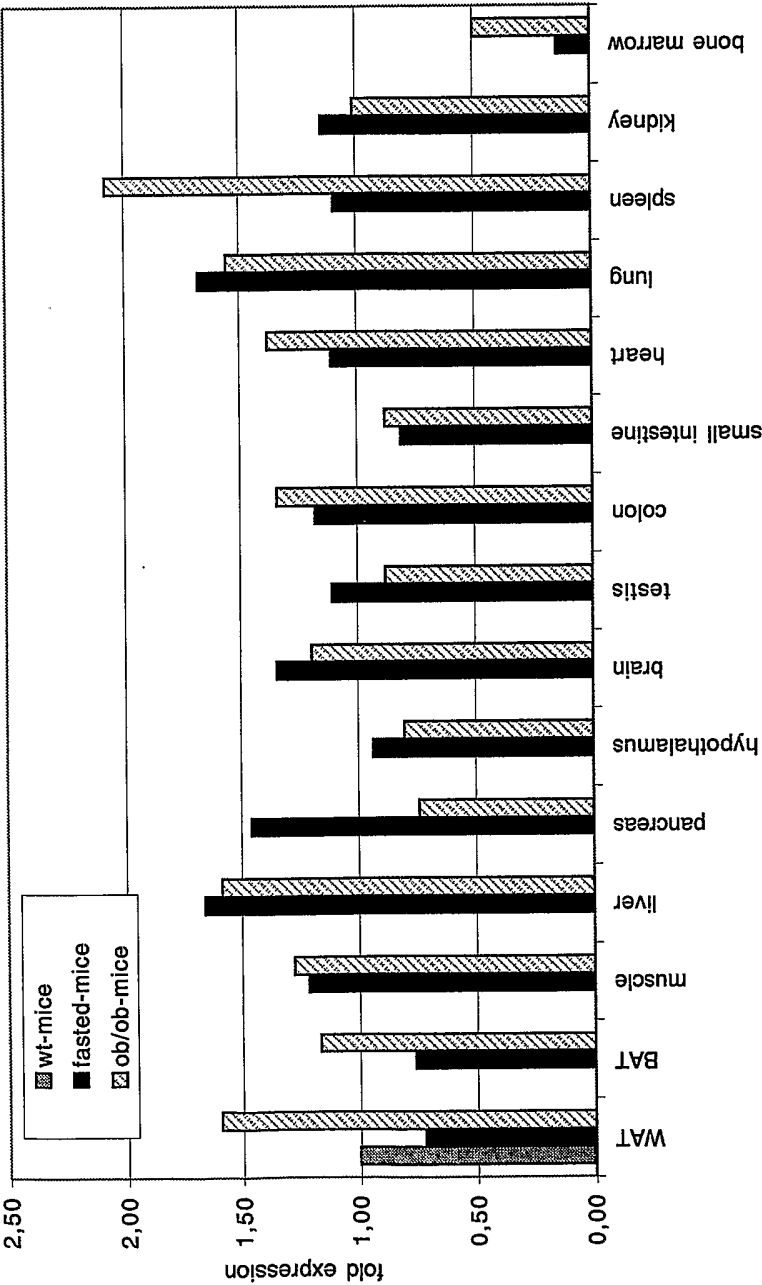


Figure 13G. Real-time PCR analysis of LOC192767 expression in mice fed with a high fat diet compared to mice fed with a standard diet

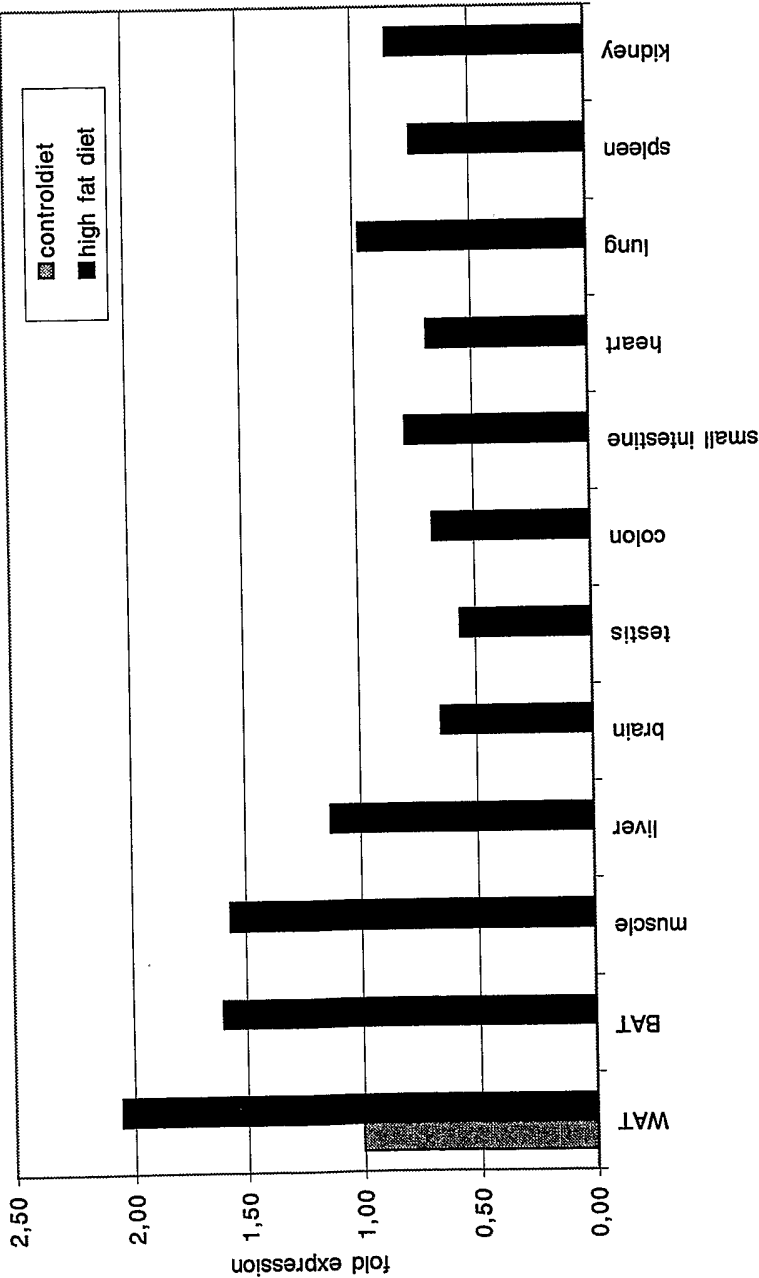


Figure 13H. Real-time PCR analysis of LOC192767 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

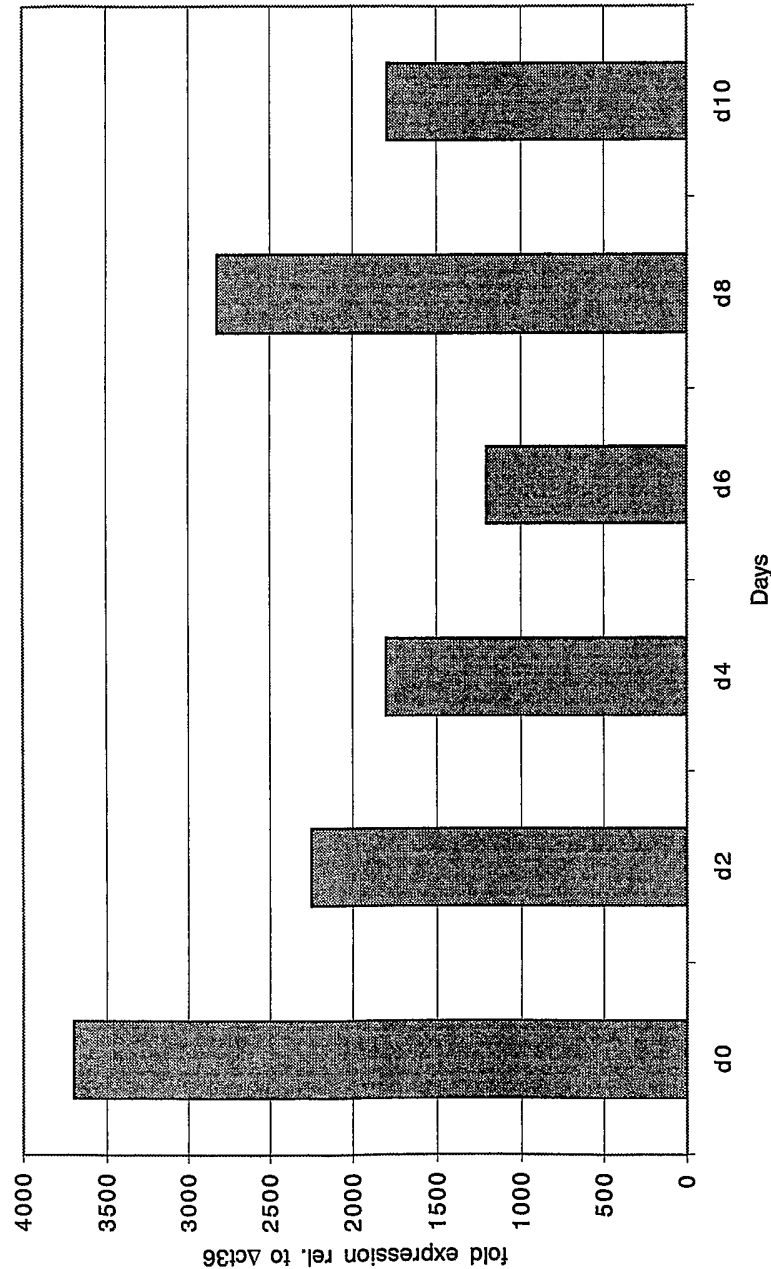


Figure 14. Triglyceride content of a *Drosophila Kinesin-like protein at 67A (Klp67A*; GadFly Accession Number CG10923) mutant

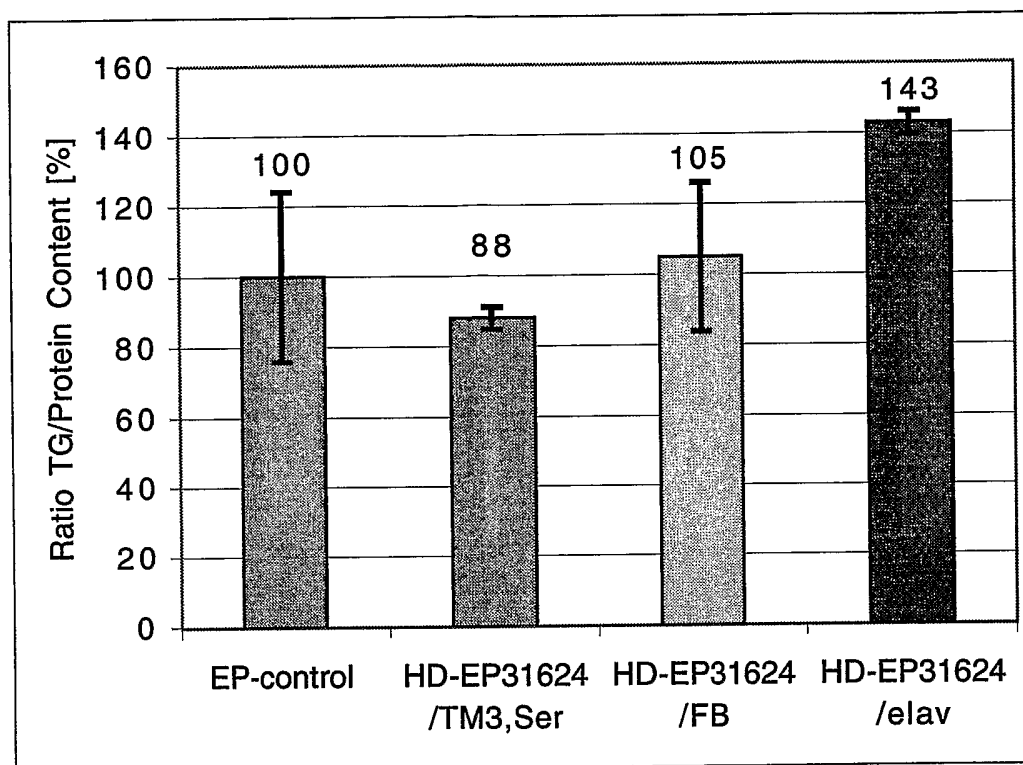


Figure 15. Molecular organization of the *Klp67A* gene (GadFly Accession Number CG10923)

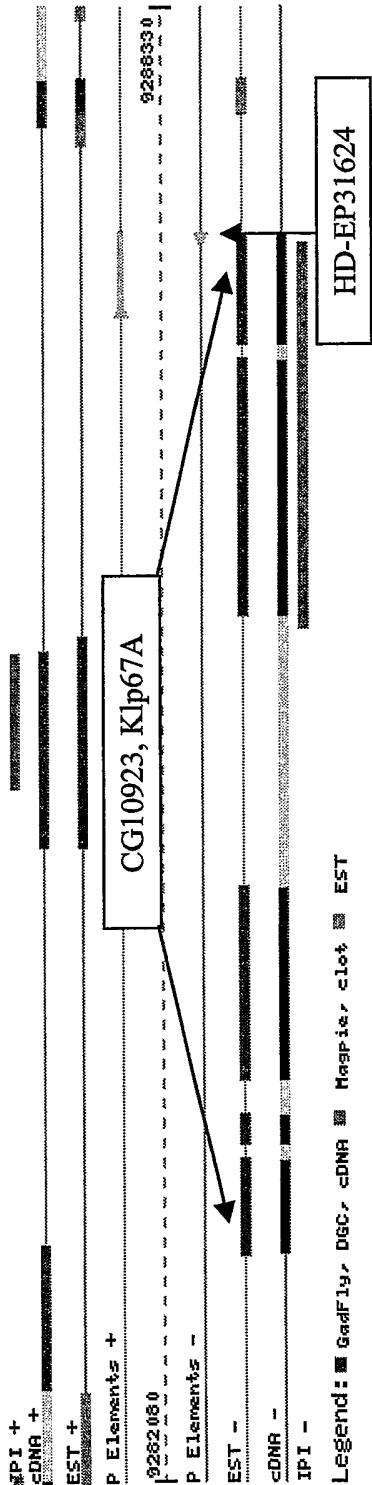


Figure 16. Expression of a *Klp67A* Homolog in Mammalian Tissues

Figure 16A. Real-time PCR analysis of kinesin family member 18A (Kif18a) expression in wild type mouse tissues

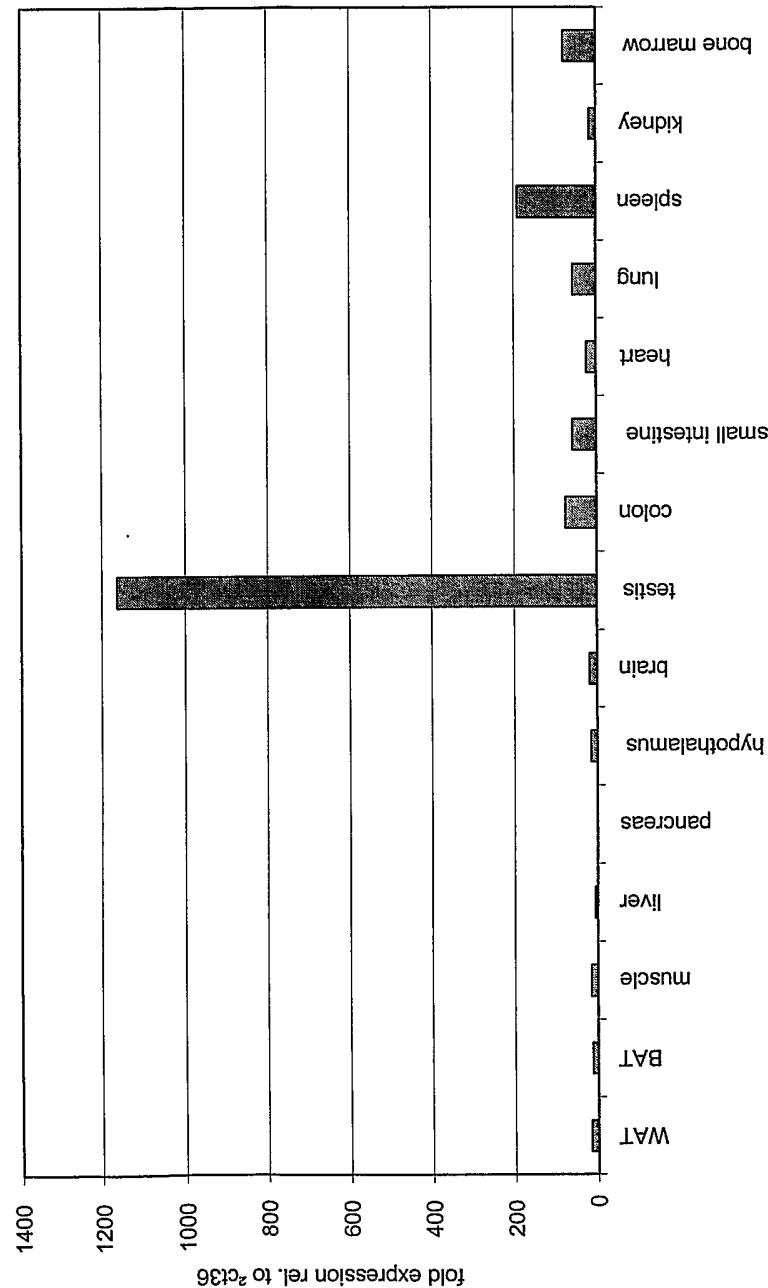


Figure 16B. Real-time PCR analysis of Kif18a expression in different mouse models

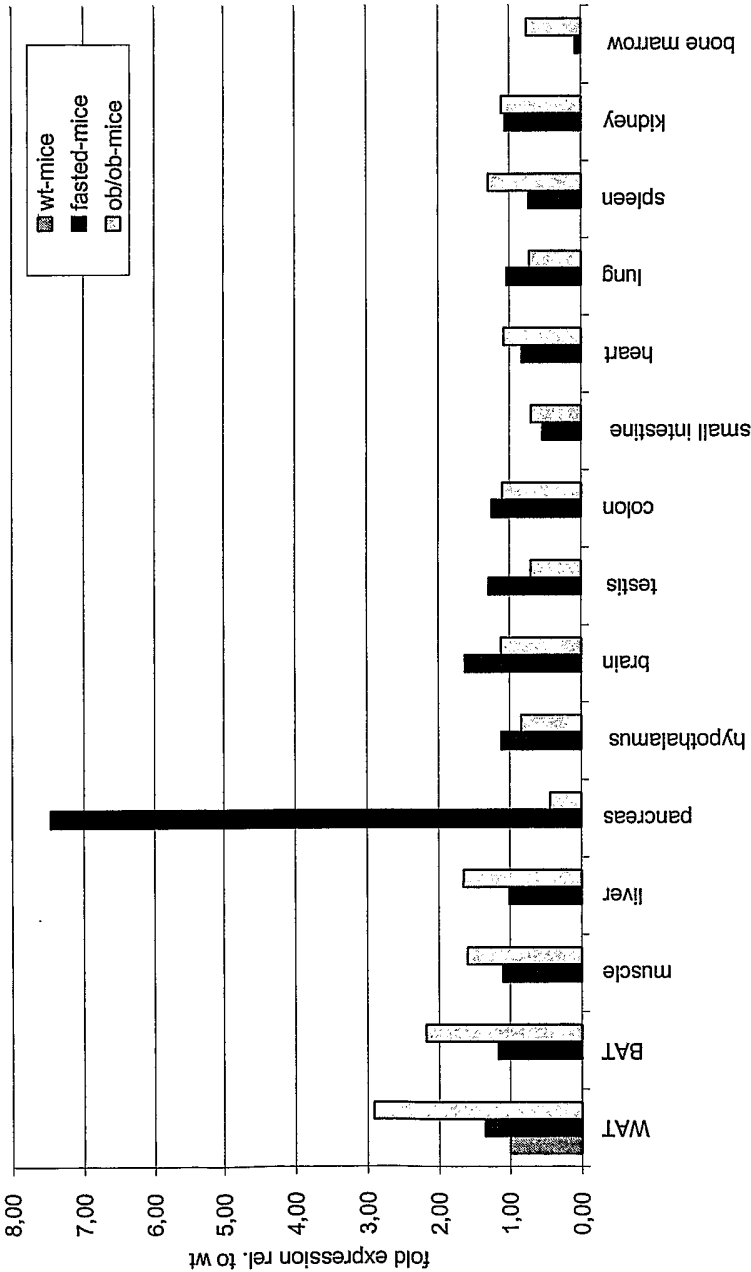


Figure 16C. Real-time PCR analysis of Kif18a expression in mice fed with a high fat diet compared to mice fed with a standard diet

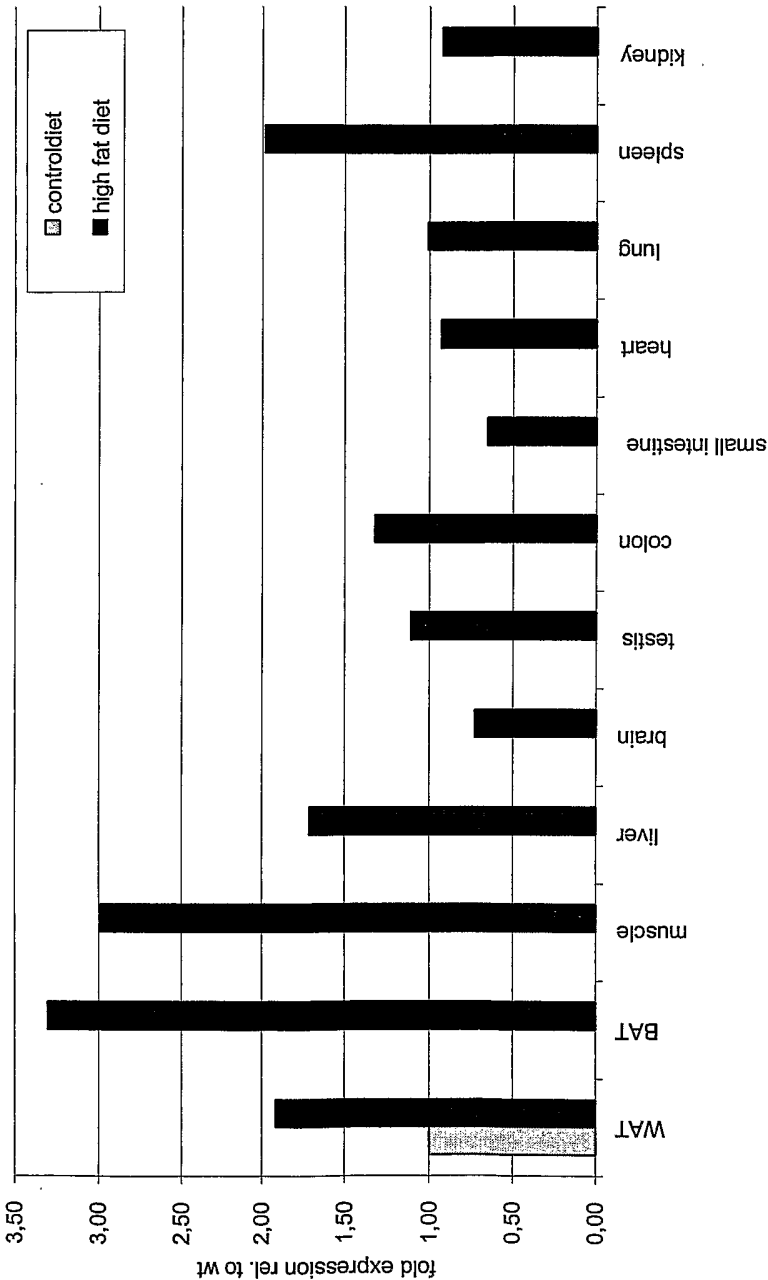


Figure 16D. Real-time PCR analysis of Kif18a expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

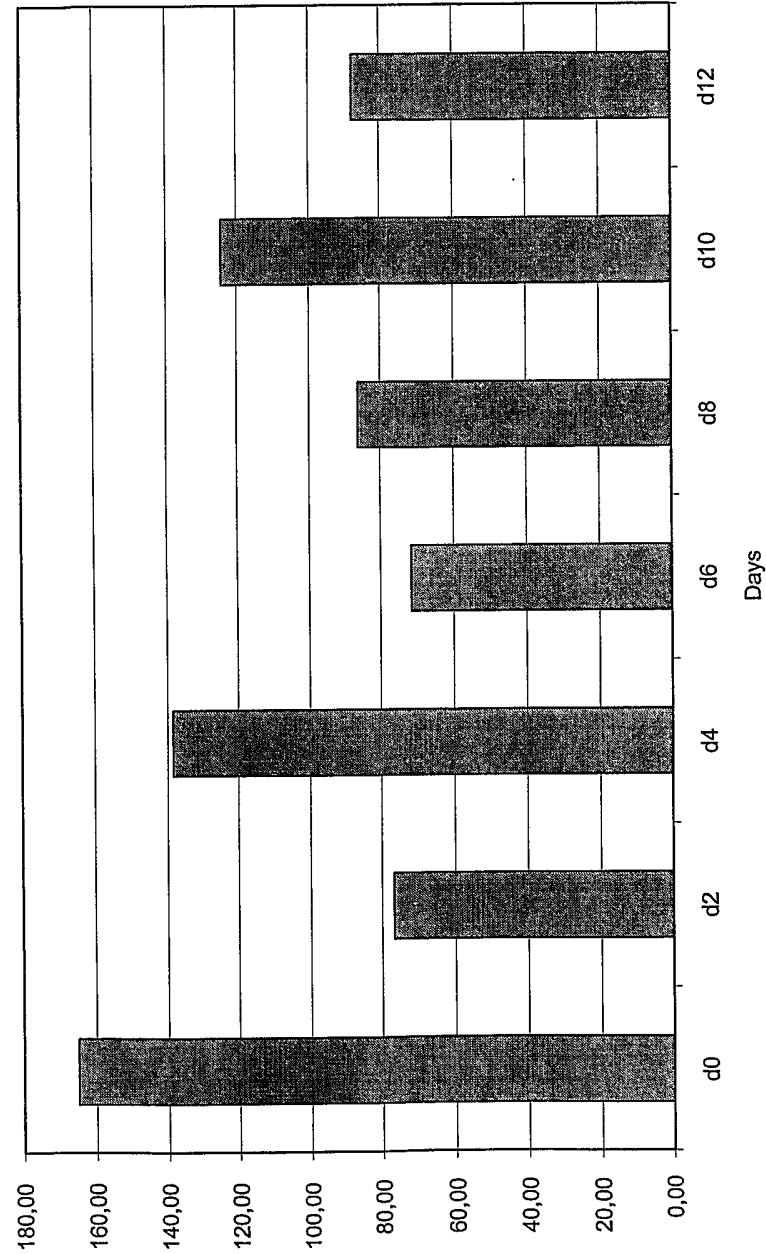


Figure 17. Triglyceride content of a *Drosophila* phospholipase A2 (GadFly Accession Number CG10133) mutant

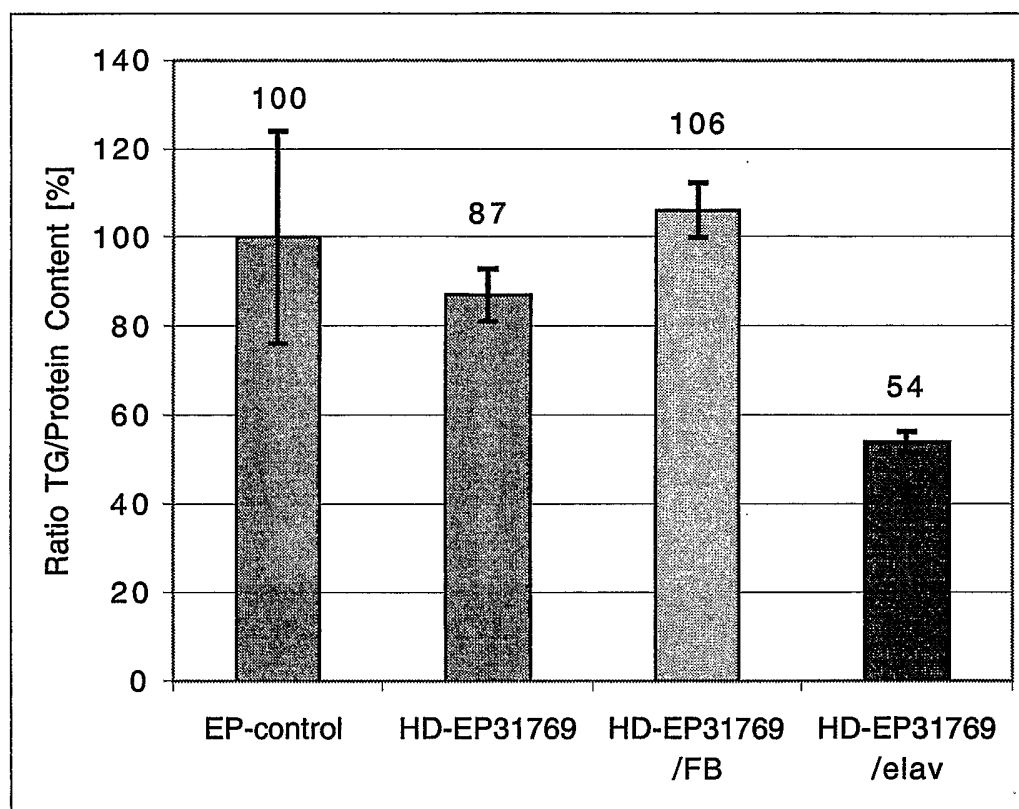


Figure 18. Molecular organization of the phospholipase A2 gene (GadFly Accession Number CG10133)

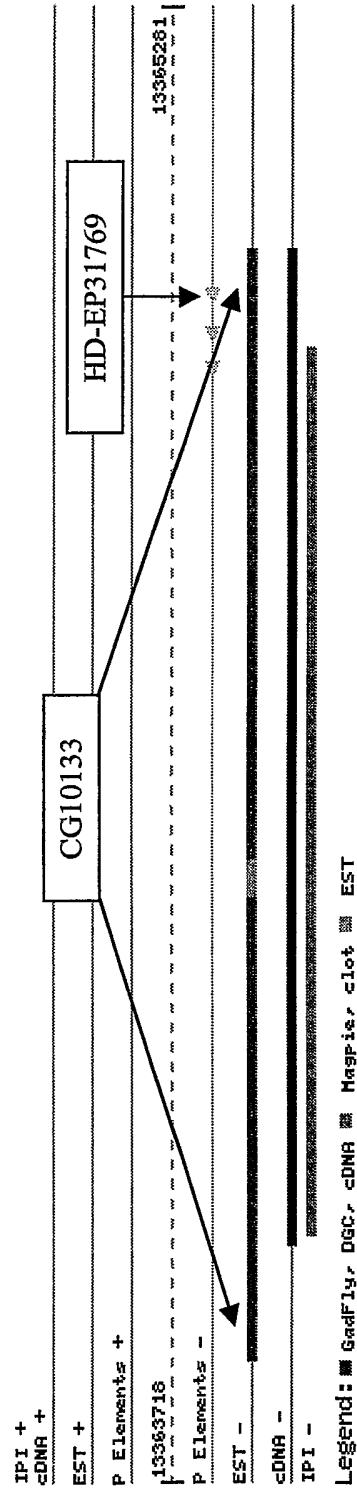


Figure 19. Expression of a CG10133 Homolog in Mammalian Tissues

Figure 19A. Real-time PCR analysis of protein similar to phospholipase A2, group IVB (cytosolic) (LOC211429) expression in wild type mouse tissues

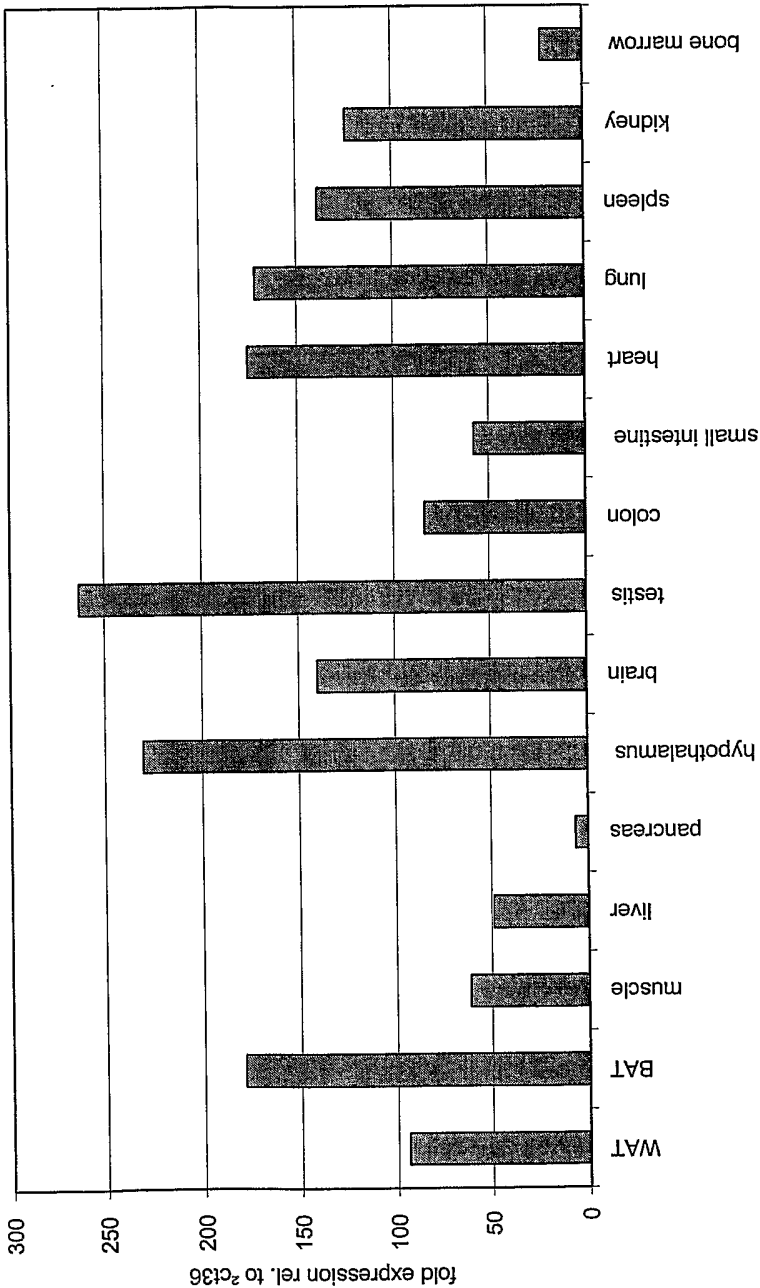


Figure 19B. Real-time PCR analysis of LOC211429 expression in different mouse models

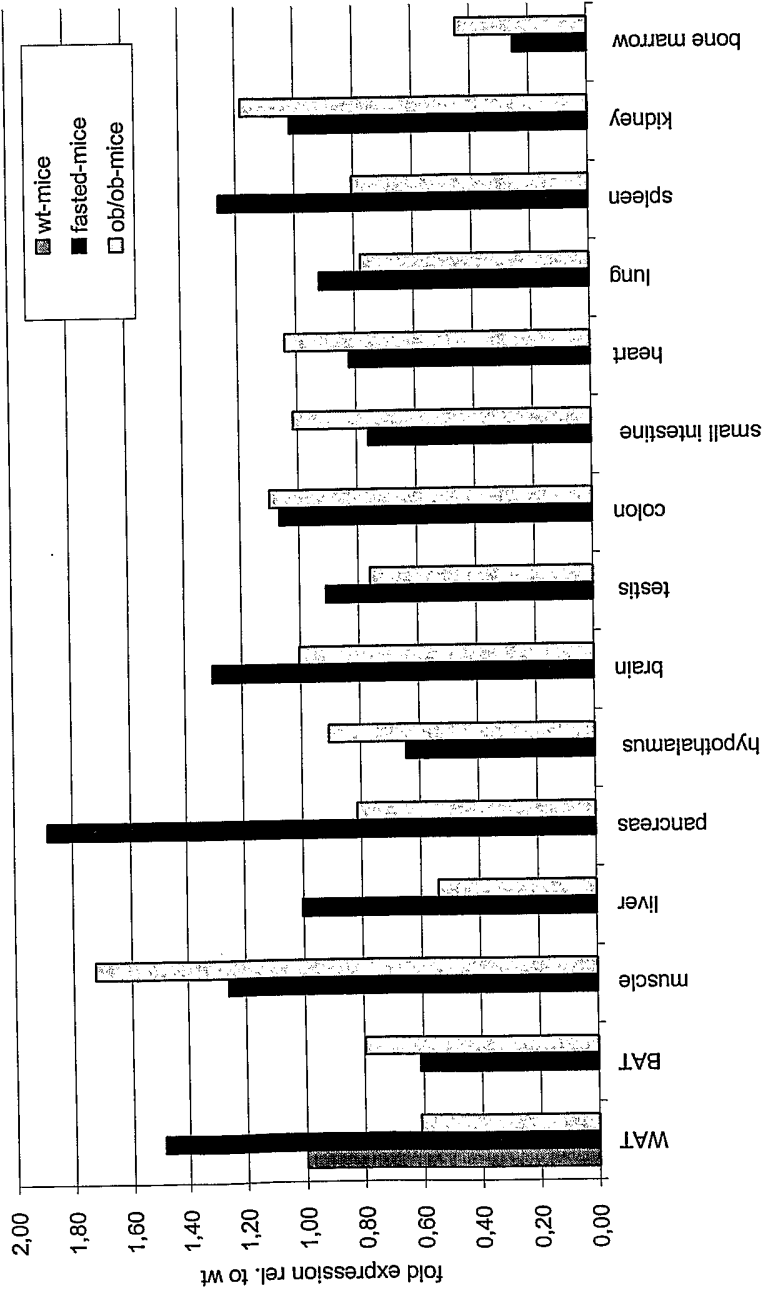


Figure 19C. Real-time PCR analysis of LOC211429 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

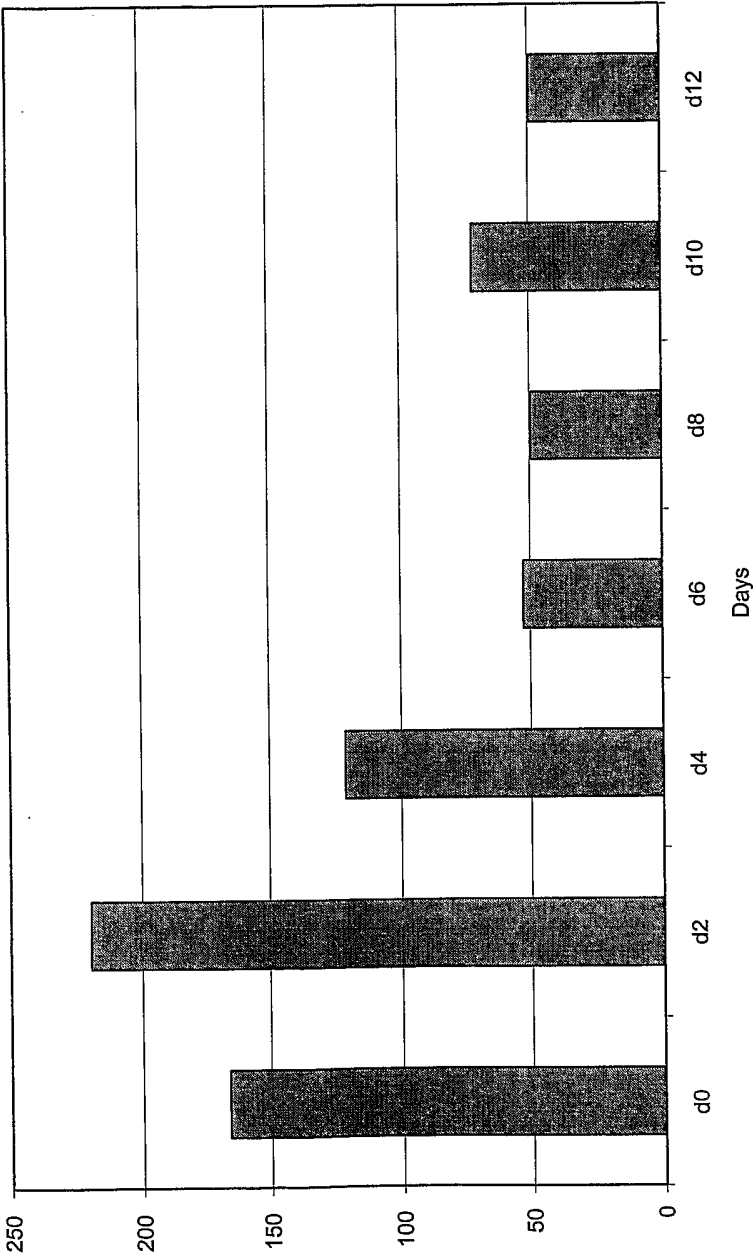


Figure 20. Triglyceride content of a *Drosophila* Acyl-CoA N-acyltransferase (GadFly Accession Number CG3967) mutant

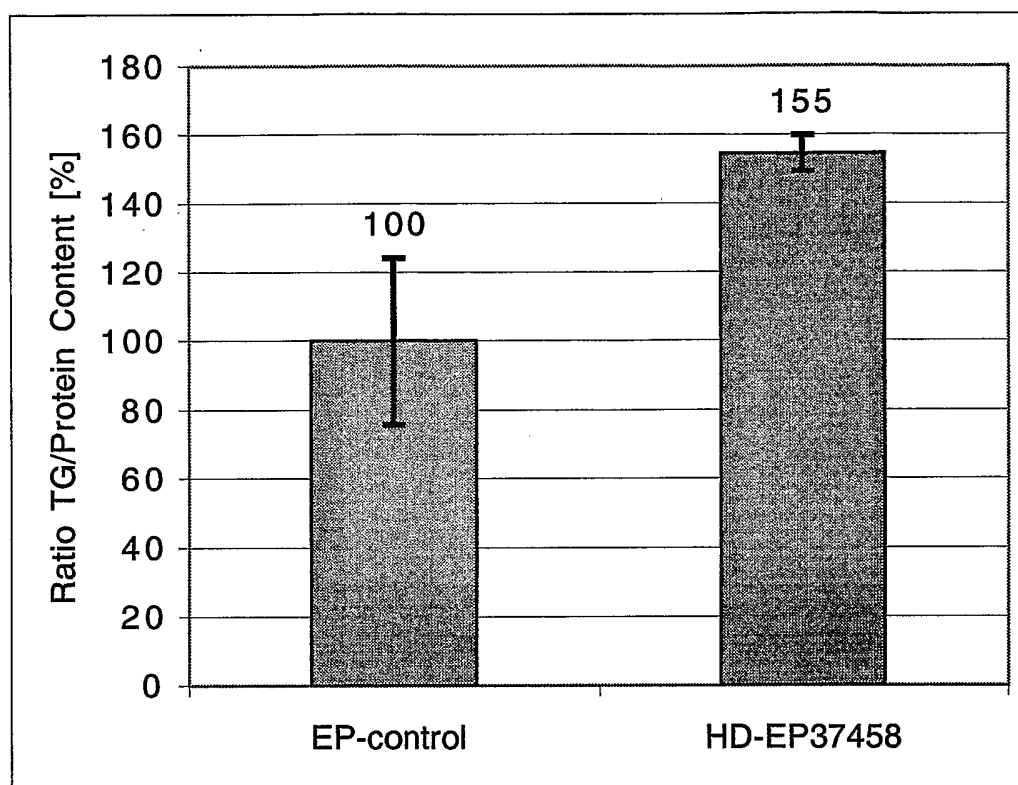


Figure 21. Molecular organization of the Acyl-CoA N-acyltransferase gene (GadFly Accession Number CG3967)

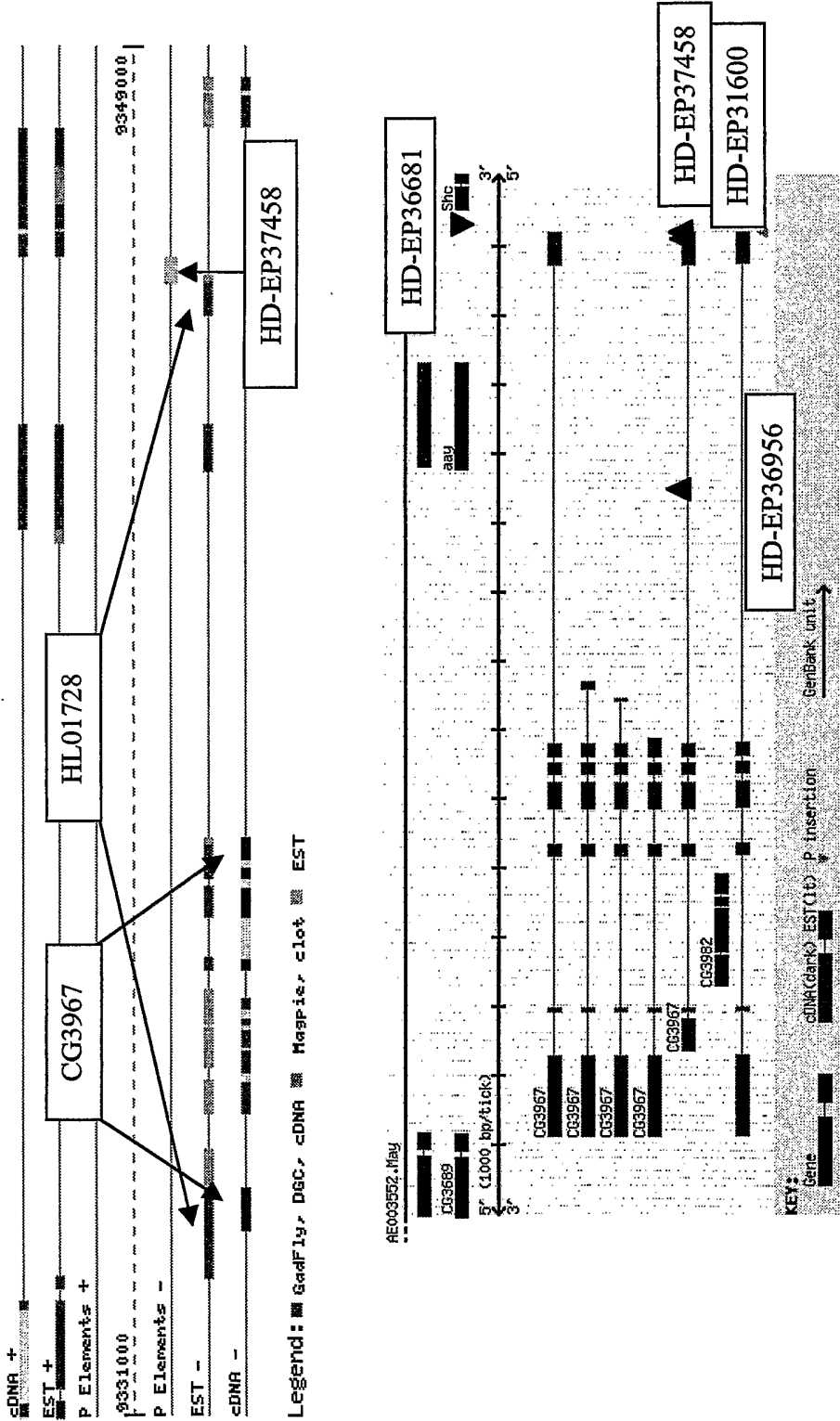


Figure 22. Triglyceride content of *Drosophila* CG32048 (GadFly Accession Number) mutants

Figure 22A.

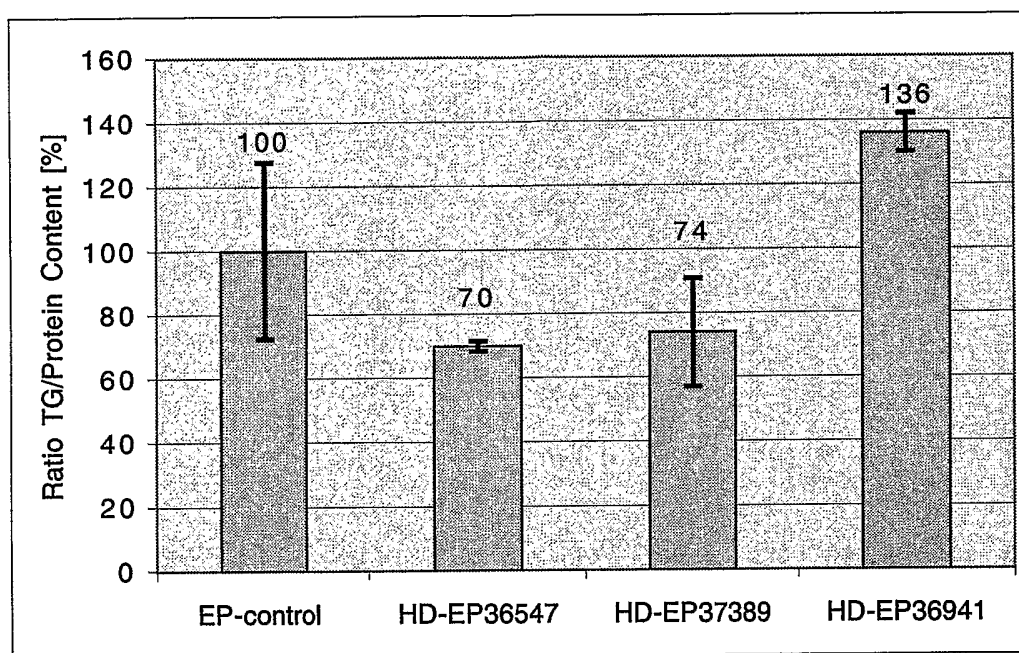


Figure 22B.

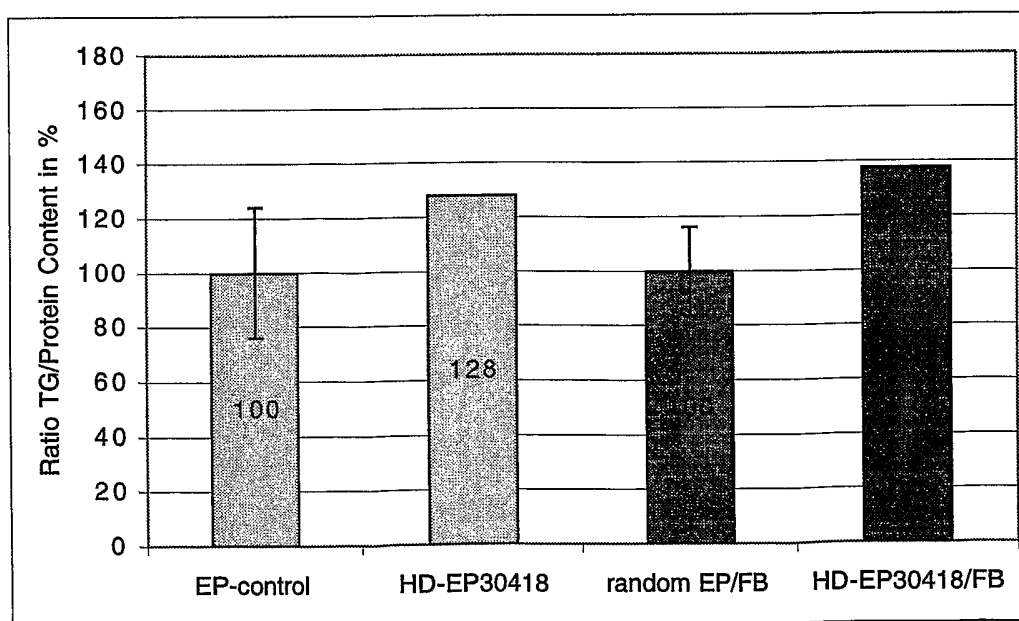


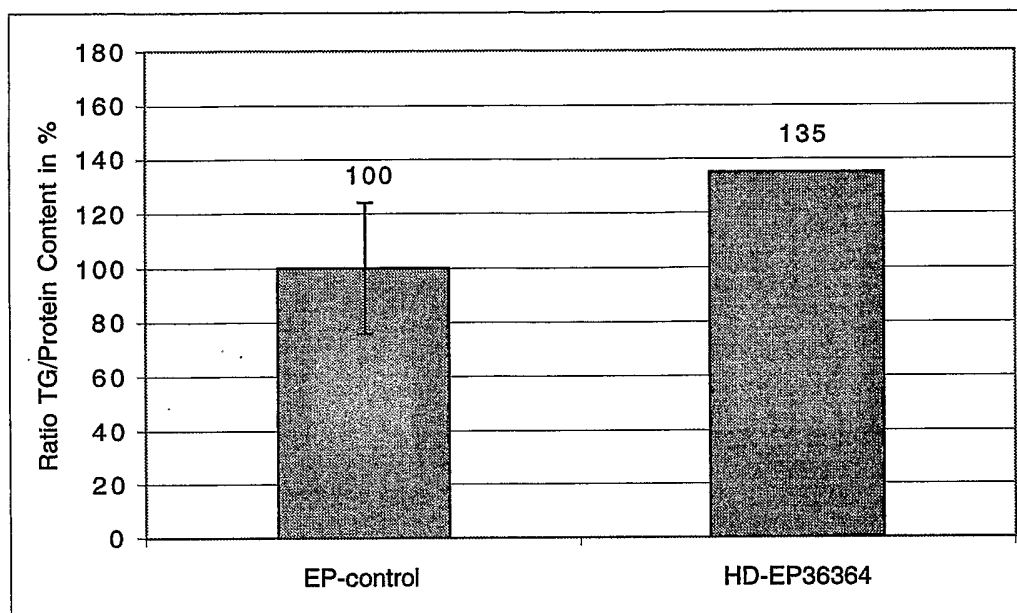
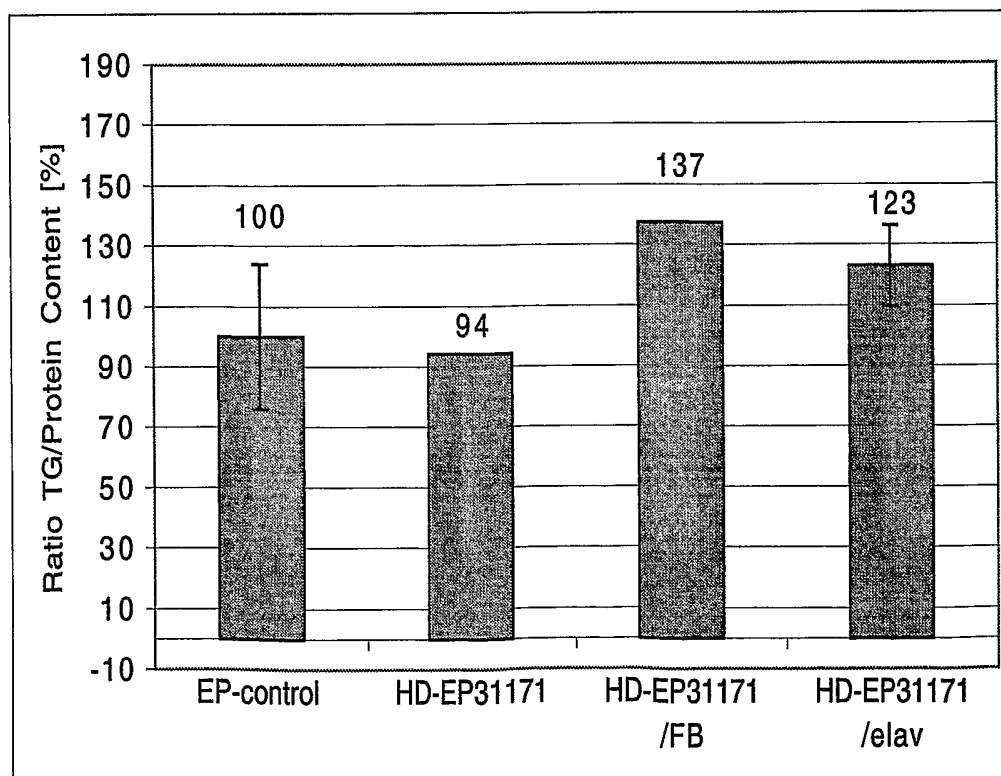
Figure 22C.**Figure 22D.**

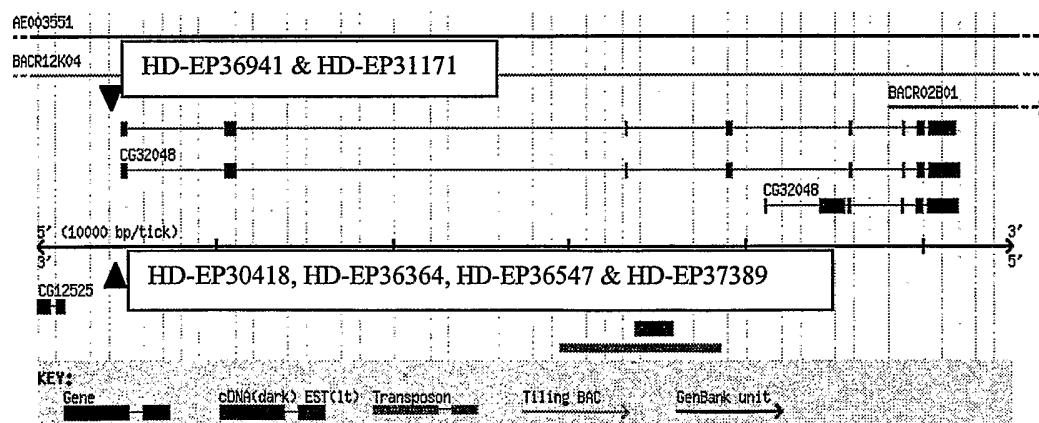
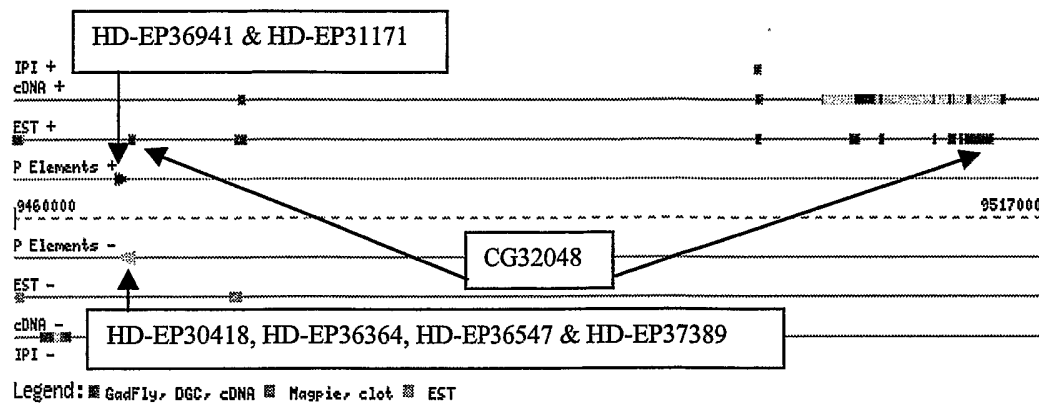
Figure 23. Molecular organisation of the CG32048 gene (GadFly Accession Number)

Figure 24. Triglyceride content of a *Drosophila* CG31605 (GadFly Accession Number) mutant

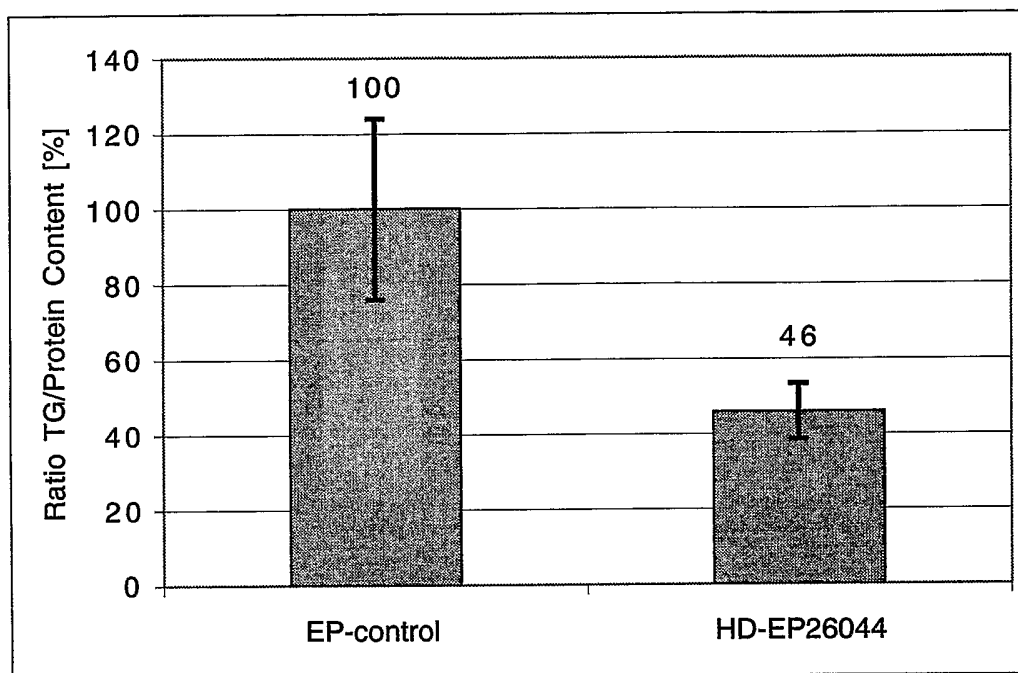


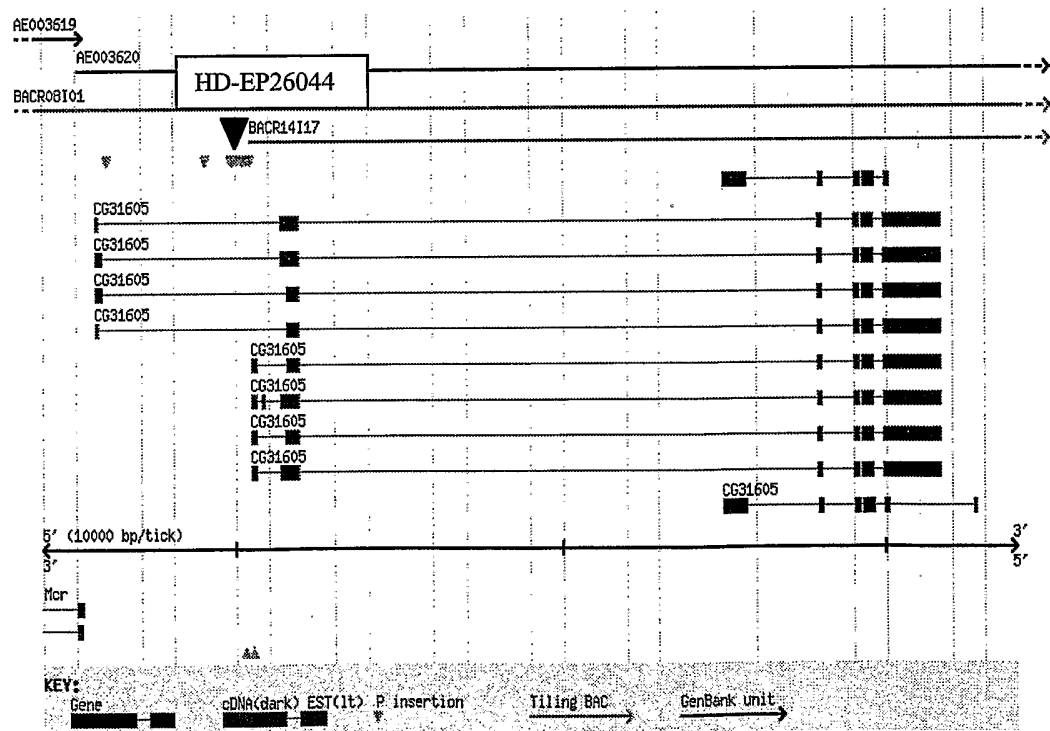
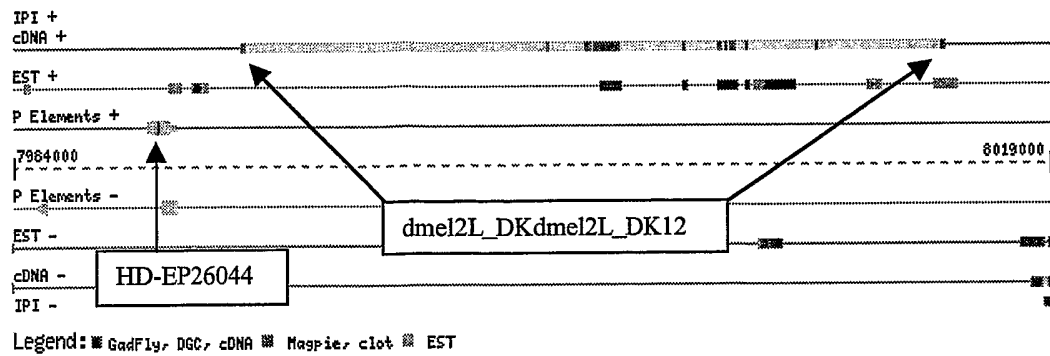
Figure 25. Molecular organization of the CG31605 gene (GadFly Accession Number)

Figure 26. Expression of human CG31605 homologs in mammalian (human) tissue
Figure 26A. Quantitative analysis of stromal cell derived factor receptor 1 (SDFR1) expression in human abdominal adipocyte cells during the differentiation from preadipocytes to mature adipocytes

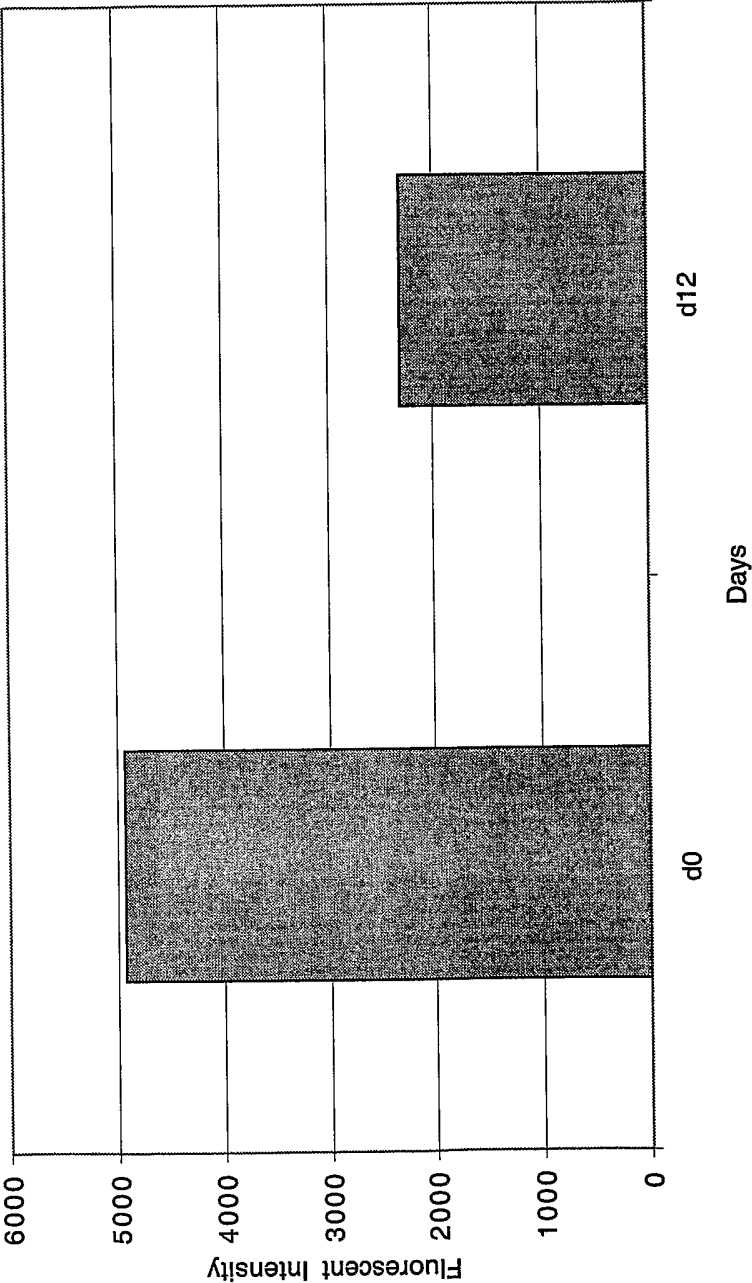


Figure 26B. Quantitative analysis of basigin (BSG) expression in human abdominal adipocyte cells during the differentiation from preadipocytes to mature adipocytes

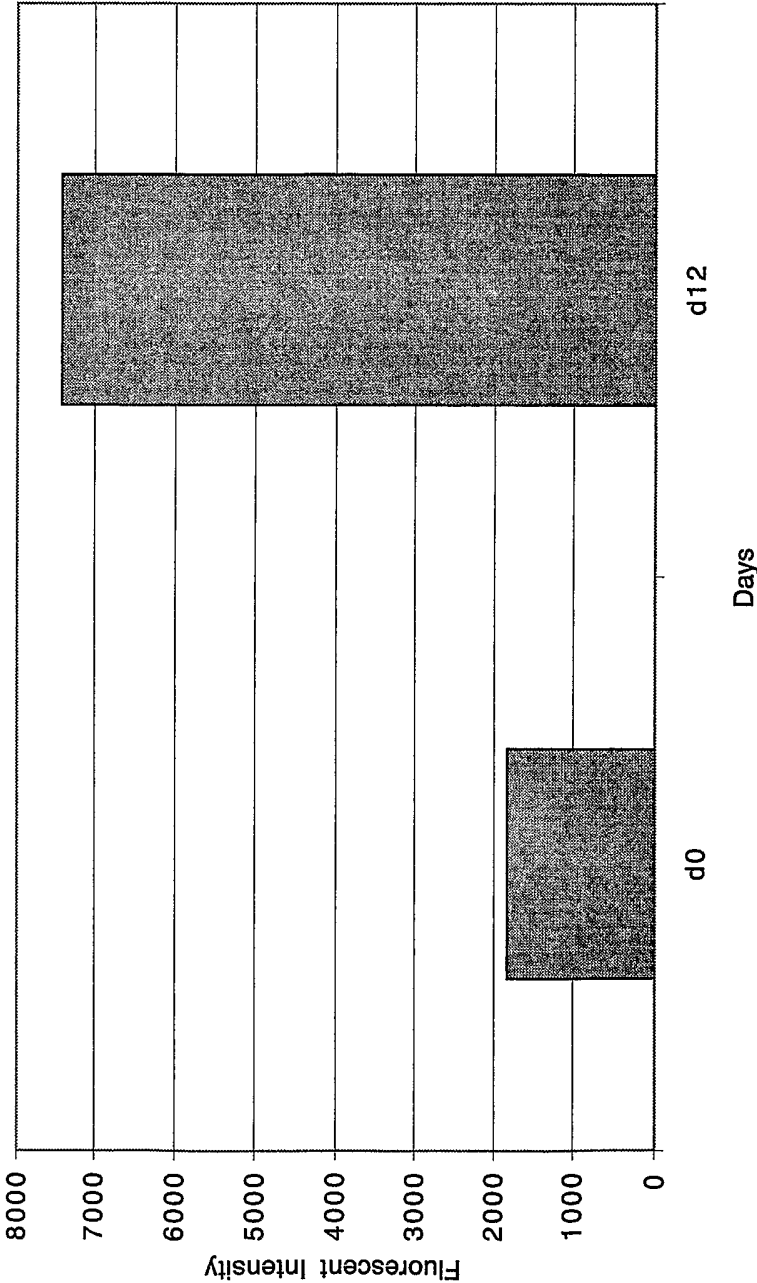


Figure 27. Triglyceride content of a *Drosophila Baldspot* (GadFly Accession Number CG3971) mutant

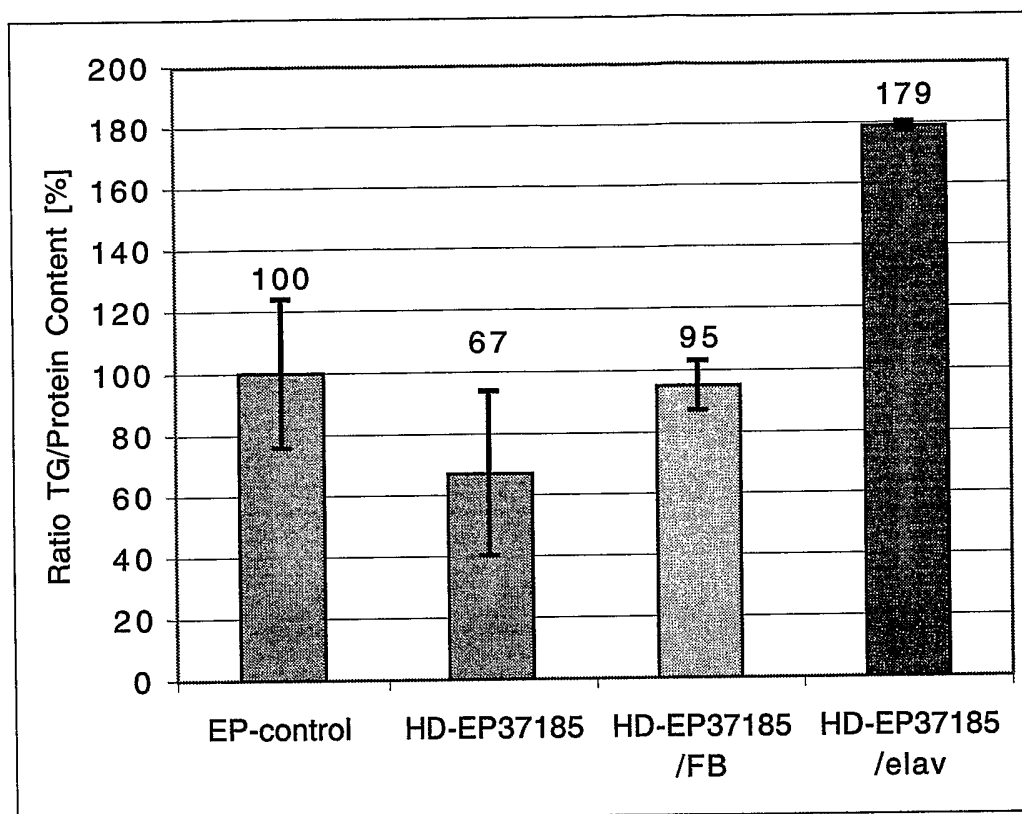


Figure 28. Molecular organisation of the *Baldspot* gene (GadFly Accession Number CG3971)

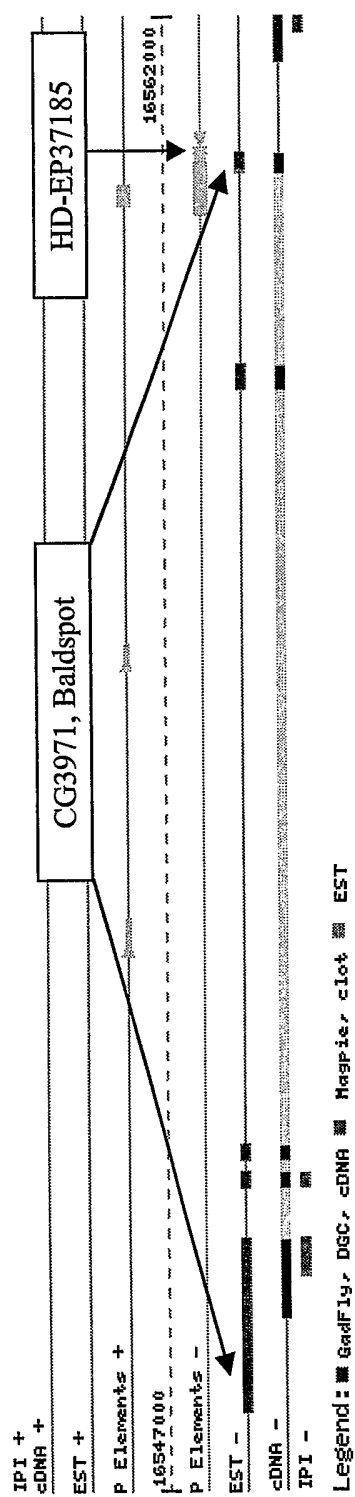


Figure 29. Expression of *Baldspot* Homologs in Mammalian Tissues

Figure 29A. Real-time PCR analysis of ELOVL family member 6, elongation of long chain fatty acids (yeast) (*Elovl6*), expression in wild type mouse tissues

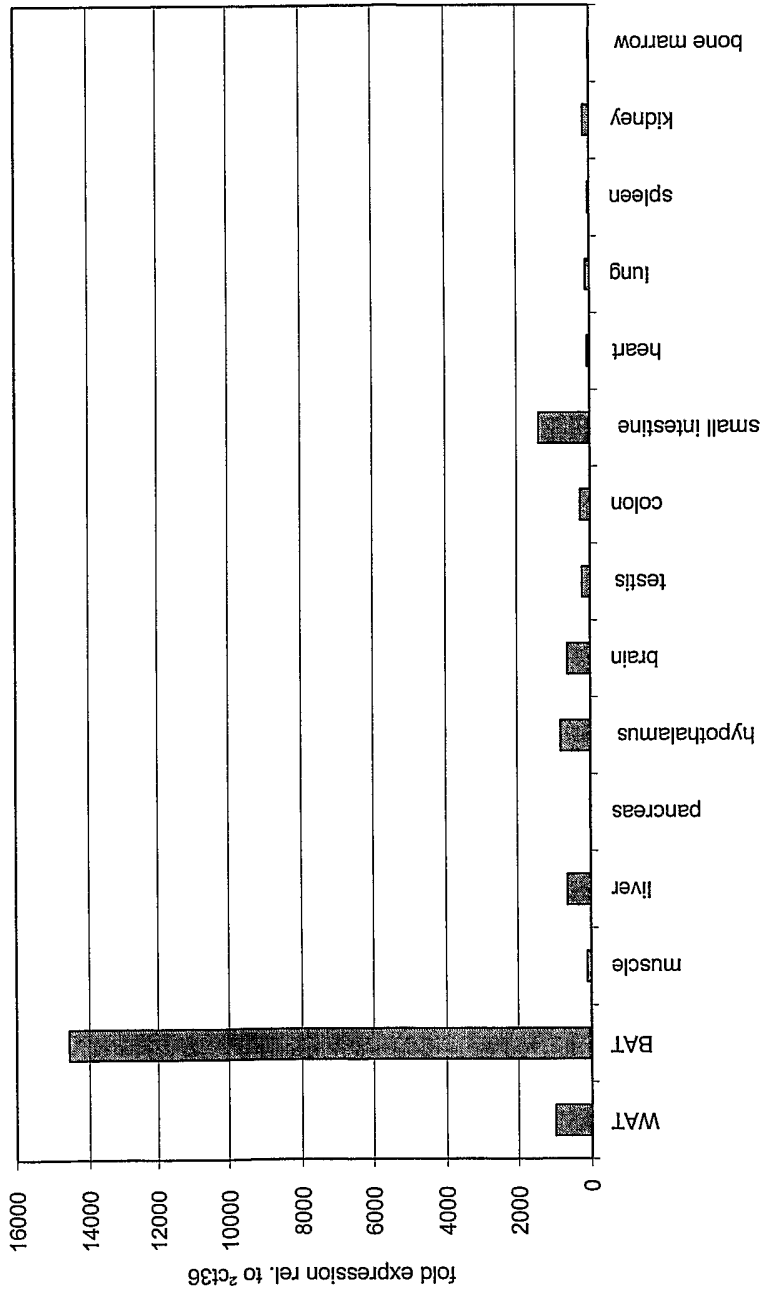


Figure 29B. Real-time PCR analysis of Elovl6 expression in different mouse models

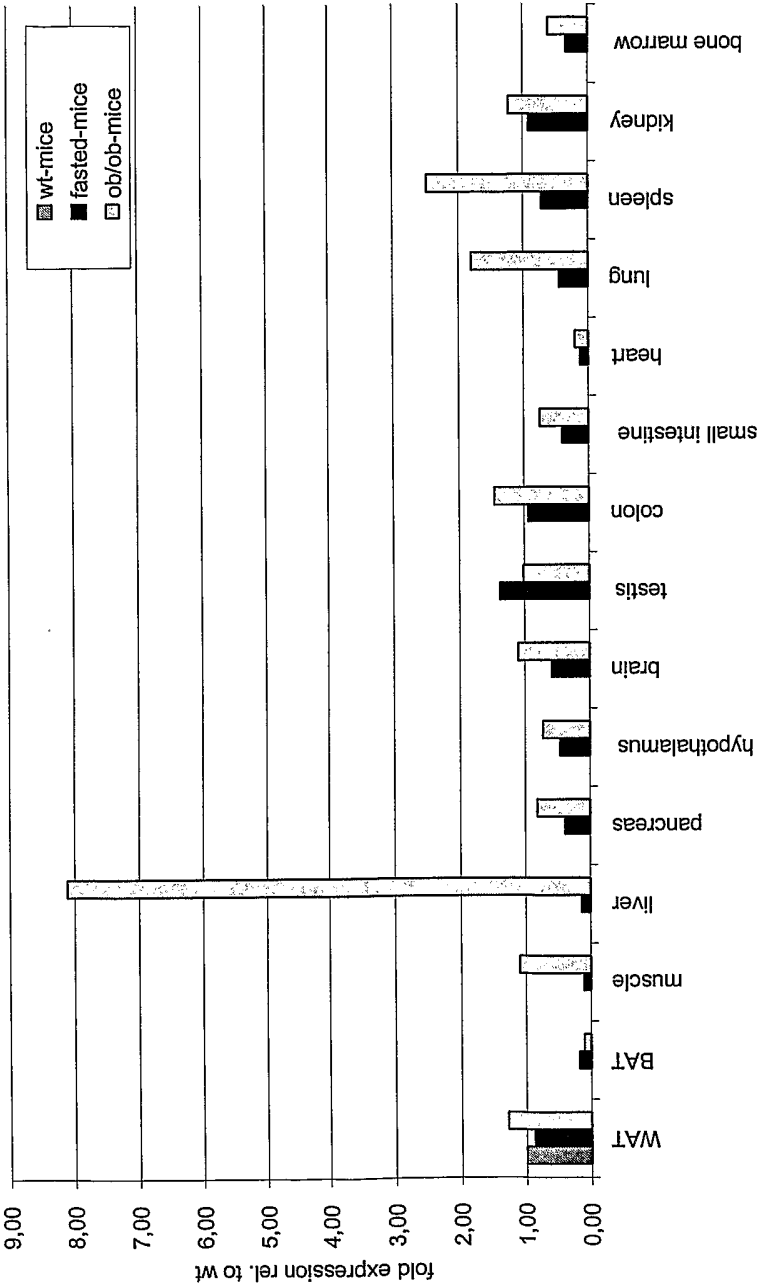


Figure 29C. Real-time PCR analysis of Elovl6 expression in mice fed with a high fat diet compared to mice fed with a standard diet

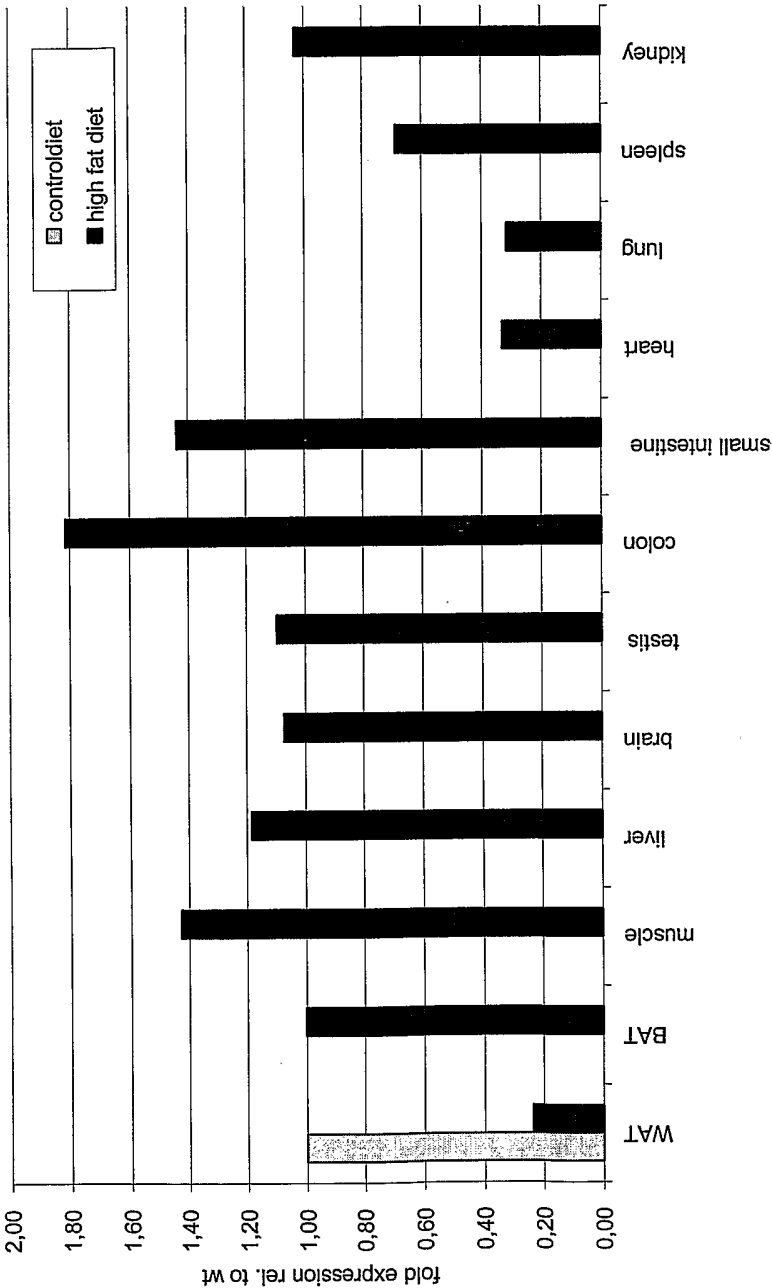


Figure 29D. Real-time PCR analysis of Elovl6 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes



Figure 29E. Real-time PCR analysis of elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3 (Elov13), expression in wild type mouse tissues

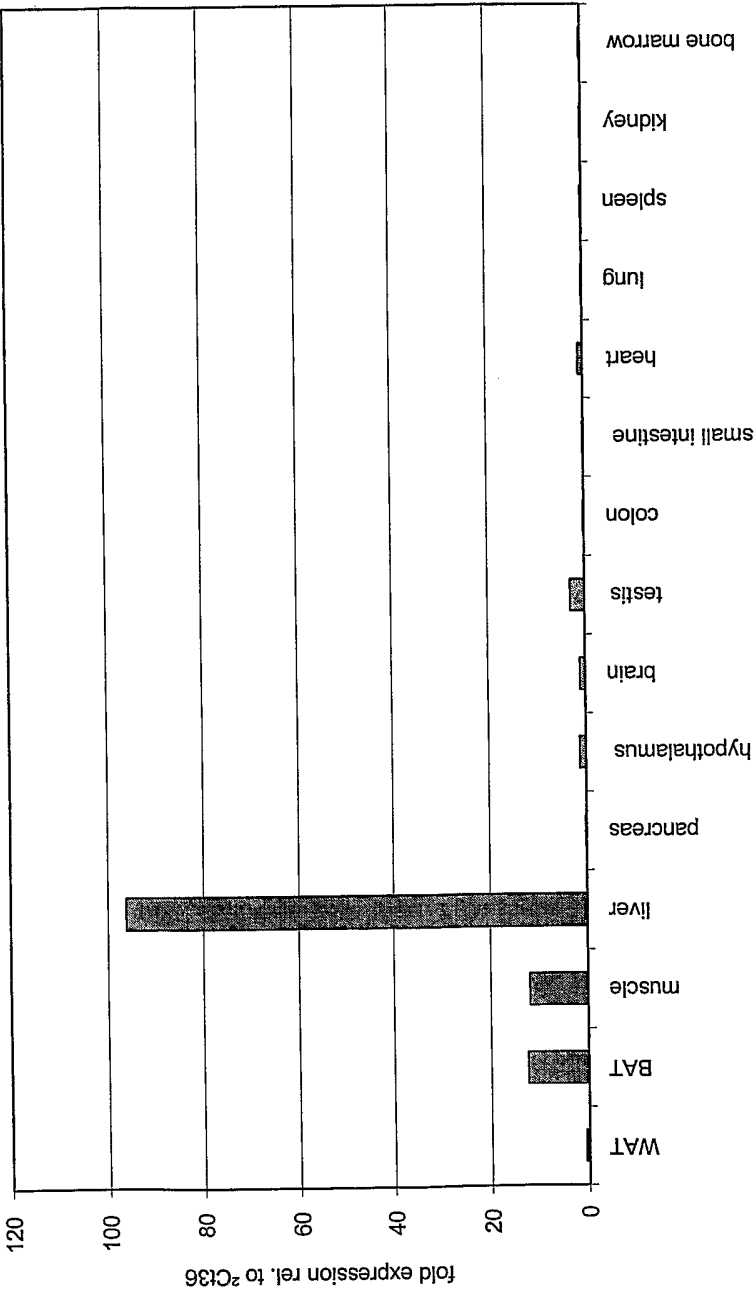


Figure 29F. Real-time PCR analysis of Elovl3 expression in different mouse models

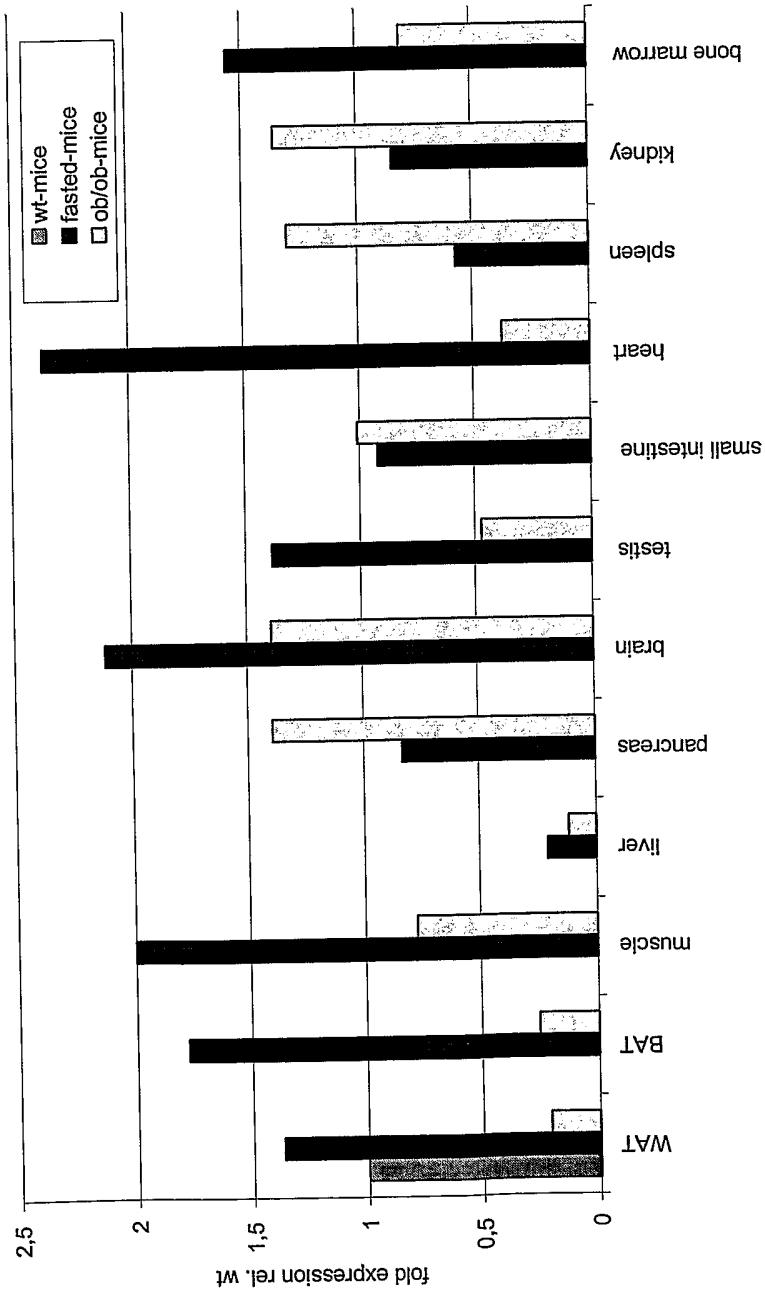


Figure 29G. Real-time PCR analysis of Elovl3 expression in mice fed with a high fat diet compared to mice fed with a standard diet

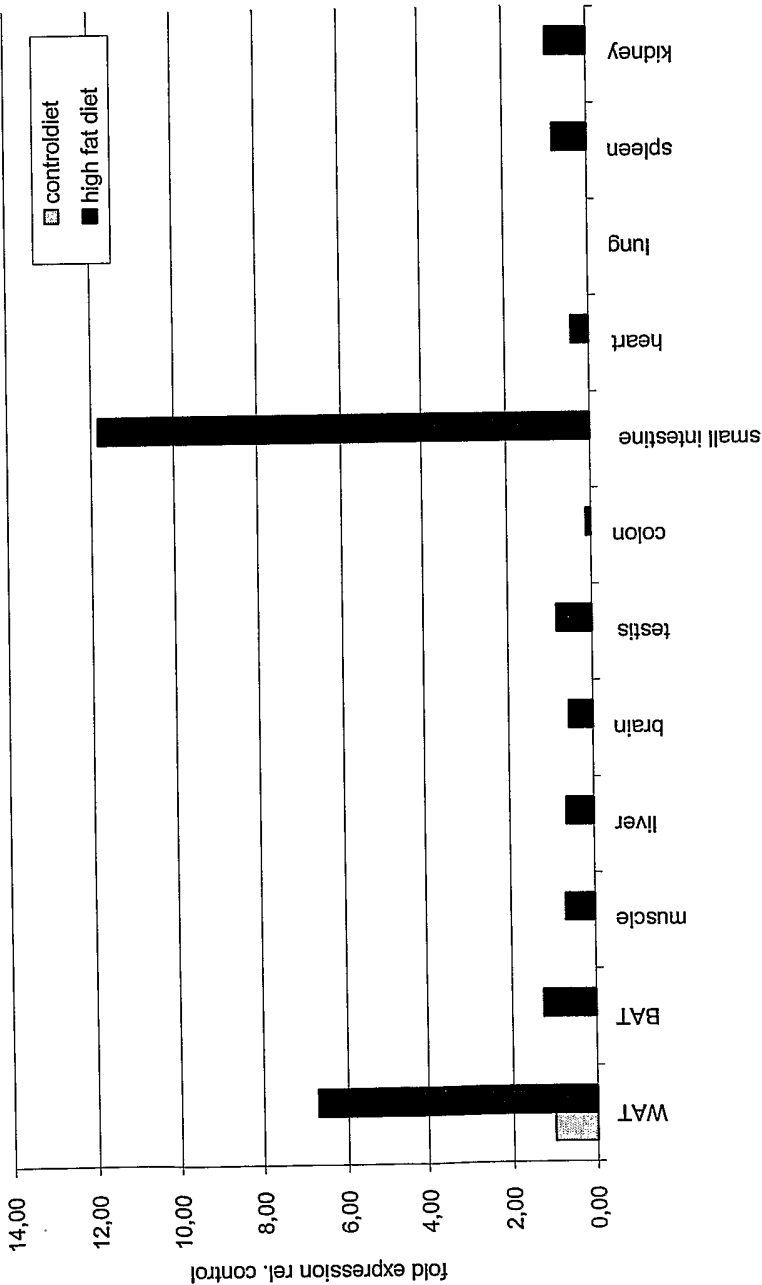


Figure 29H. Real-time PCR analysis of Elovl3 expression in 3T3-L1 cells differentiated from preadipocytes to mature adipocytes

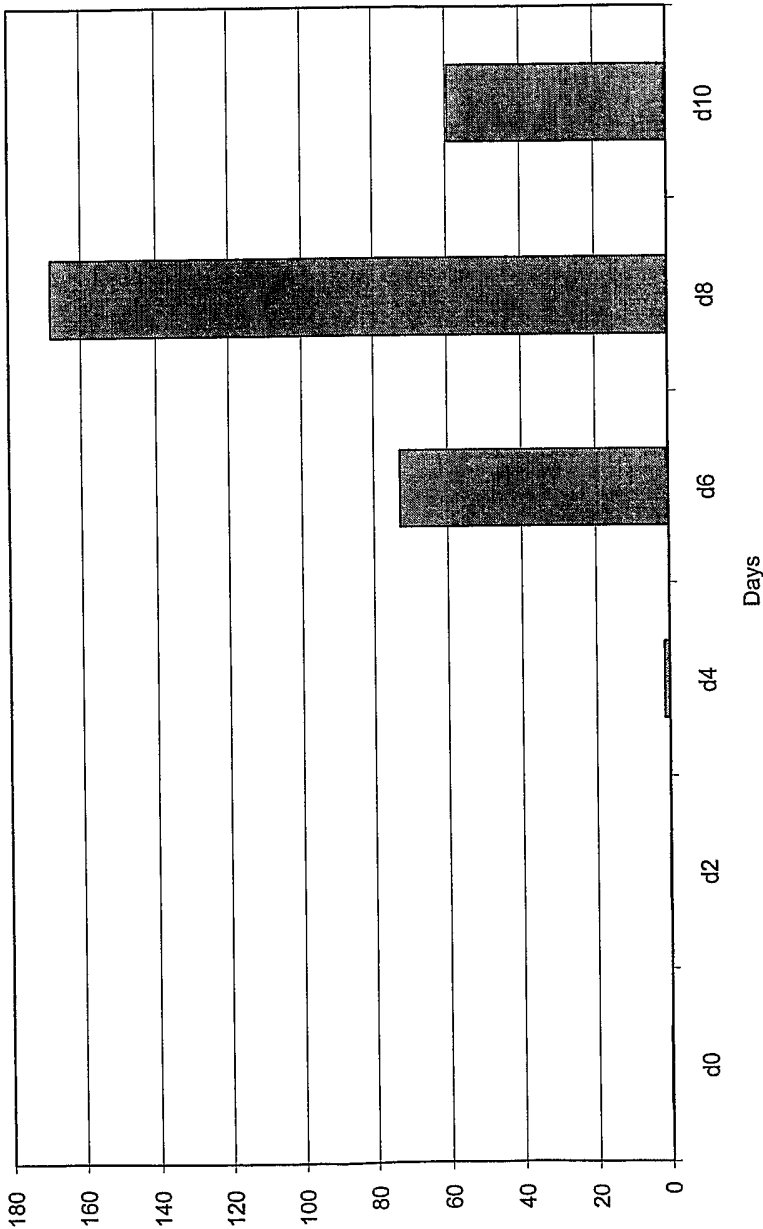


Figure 30. Expression of a human *Baldspot* homolog in mammalian (human) tissue. Quantitative analysis of ELOVL family member 6, elongation of long chain fatty acids (ELOVL6) expression in human abdominal adipocyte cells during the differentiation from preadipocytes to mature adipocytes

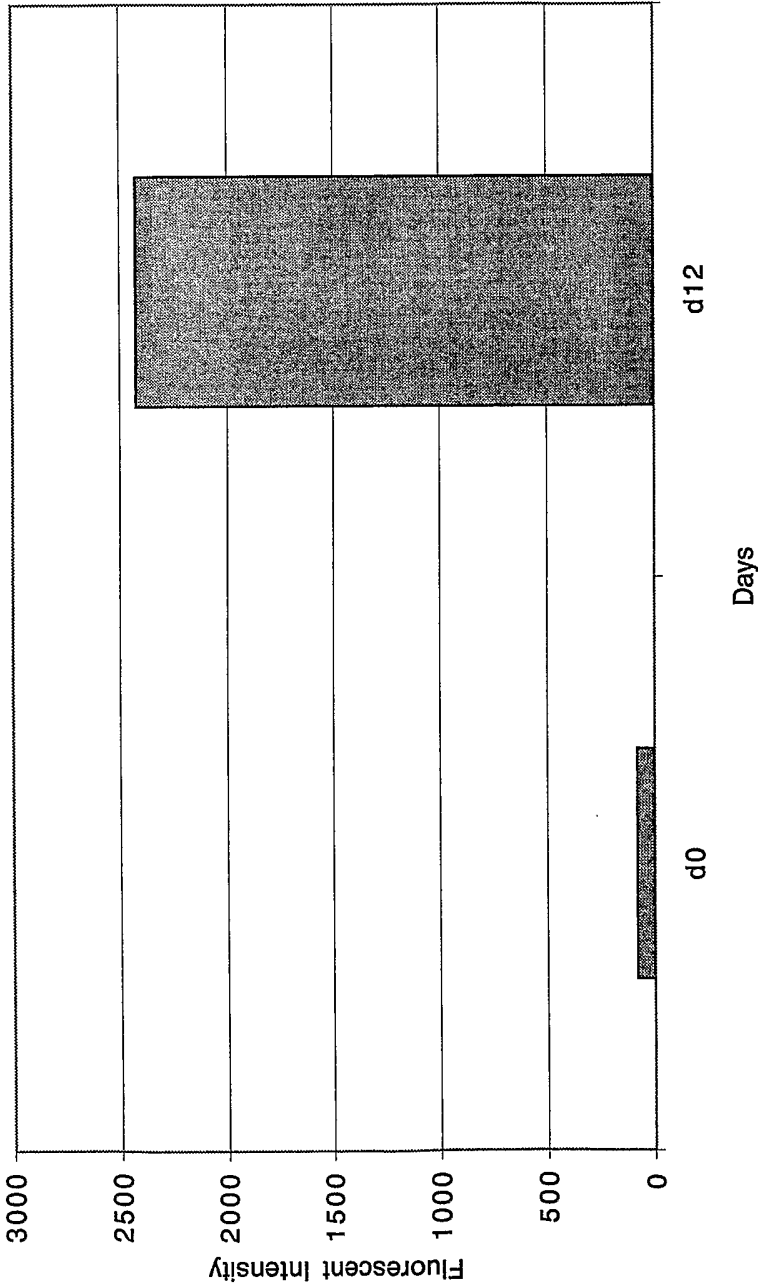


Figure 31. Triglyceride content of a *Drosophila* CG13625 (GadFly Accession Number) mutant

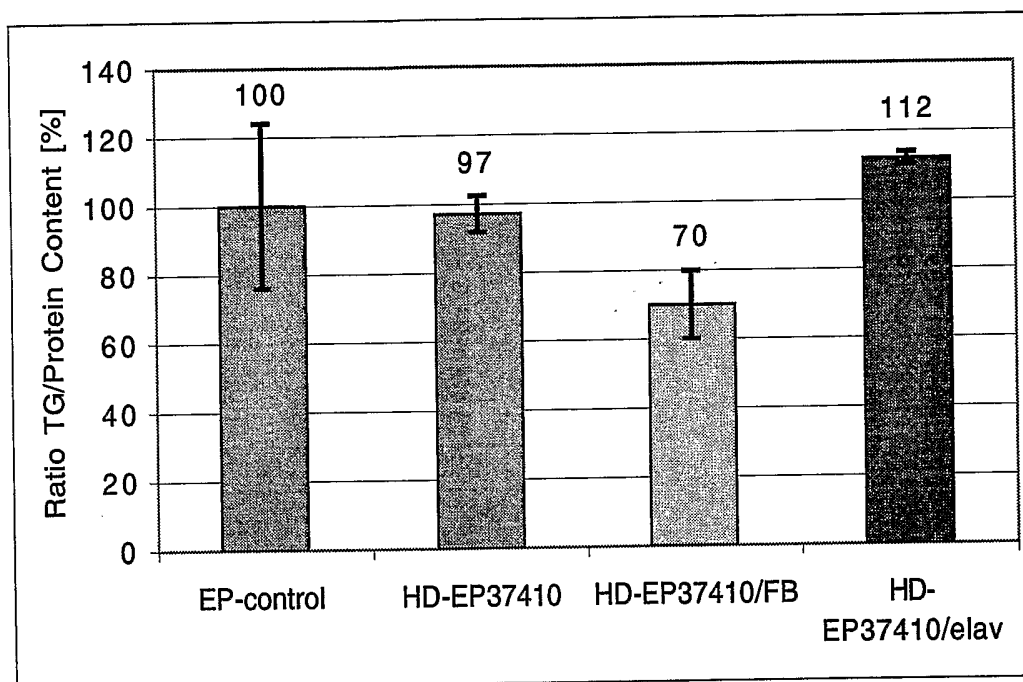


Figure 32. Molecular organization of the CG13625 gene (GadFly Accession Number)

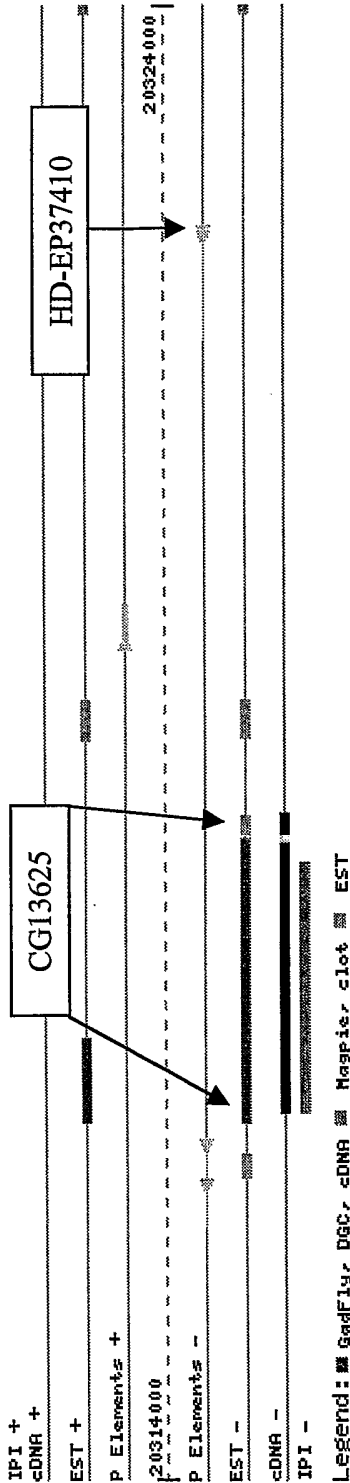


Figure 33. Triglyceride content of a *Drosophila fusilli* (GadFly Accession Number CG8205) mutant

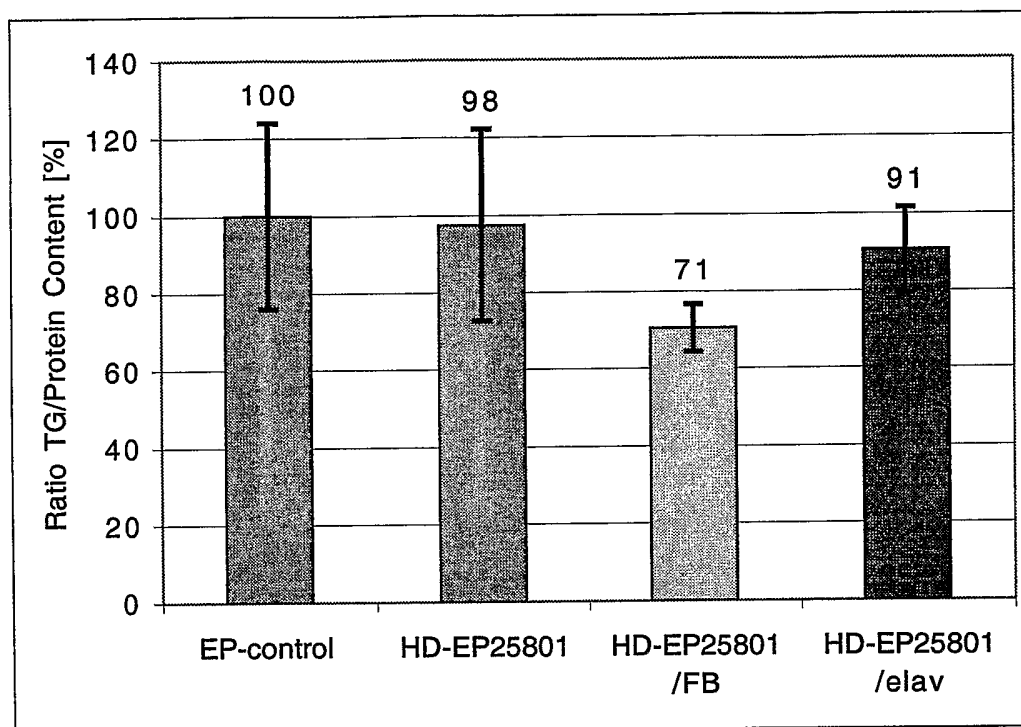


Figure 34. Molecular organization of the *fusilli* gene (GadFly Accession Number CG8205)

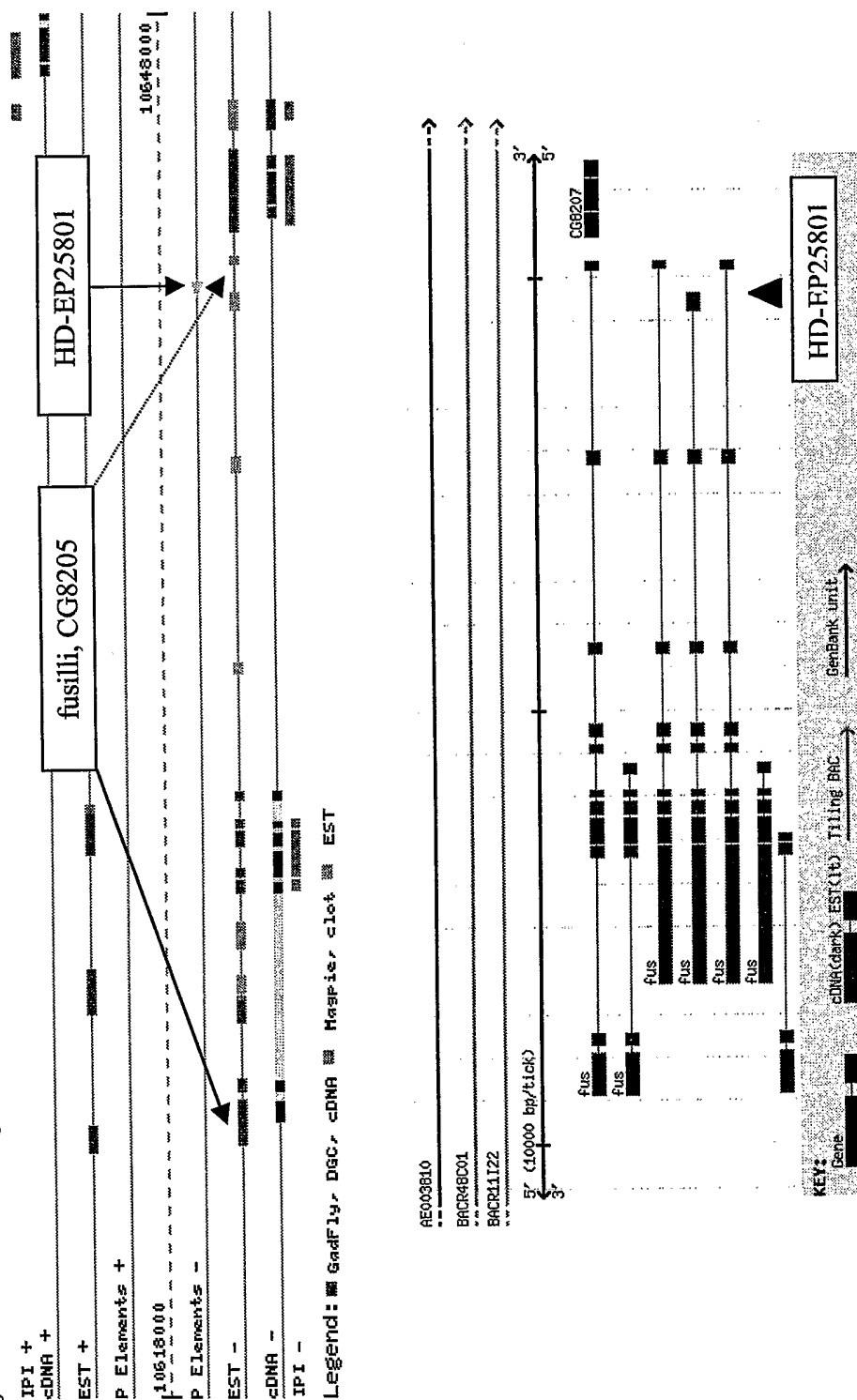


Figure 35. Triglyceride content of *Drosophila schnurri* (*shn*; GadFly Accession Number CG7734) mutants

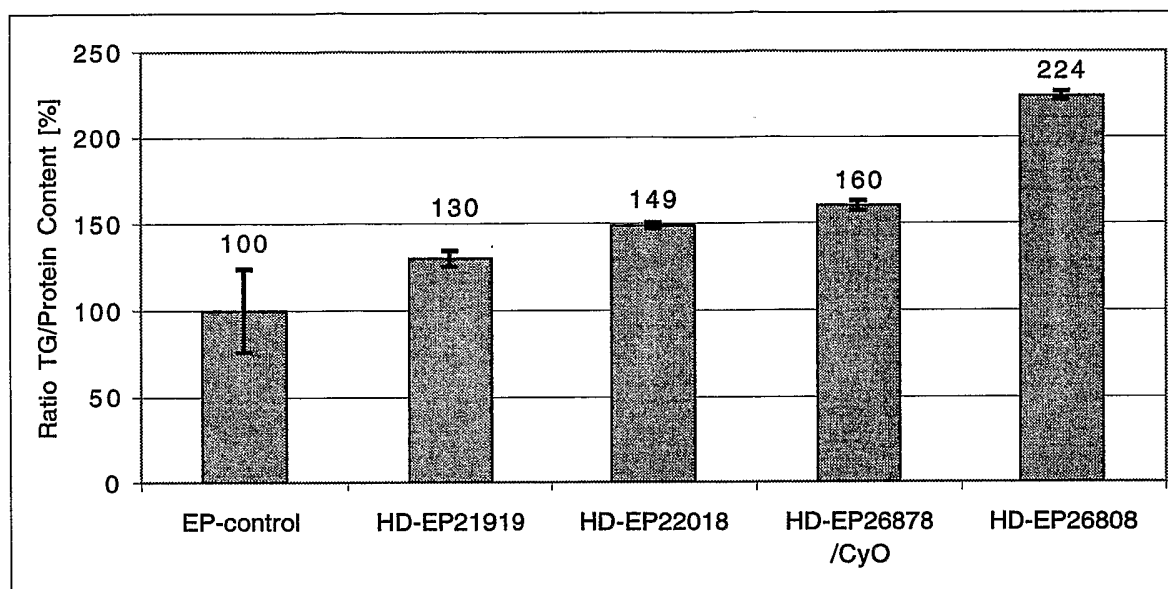


Figure 36. Molecular organisation of the *schnurri* gene (GadFly Accession Number CG7734)

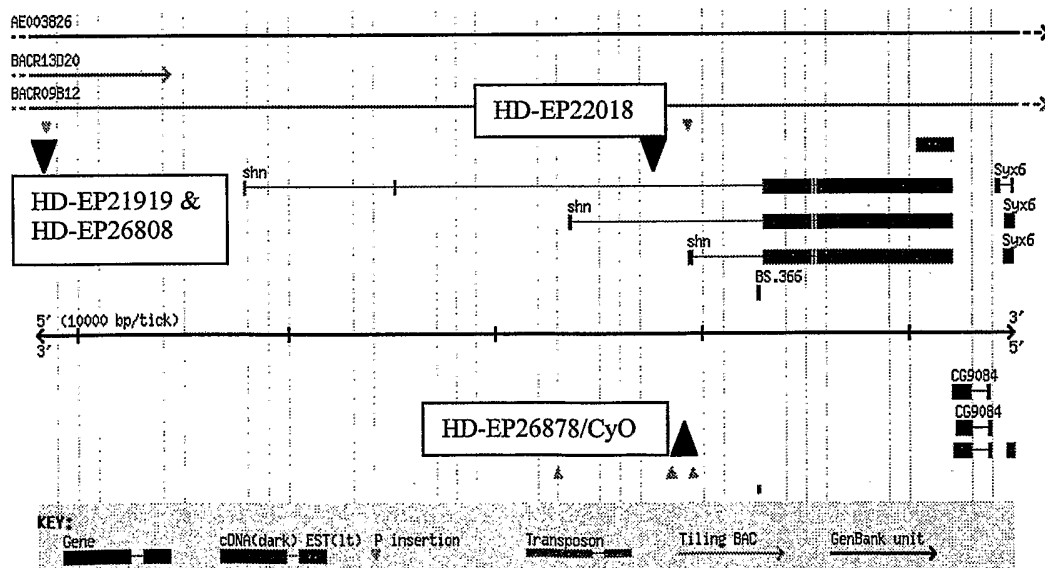
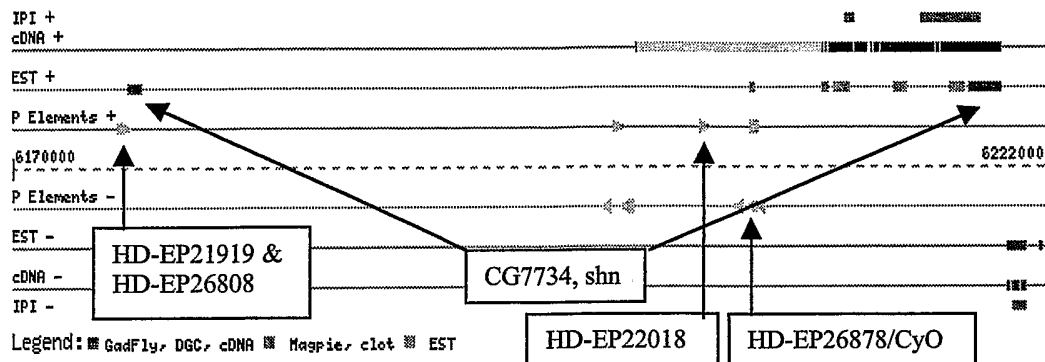


Figure 37. Expression of a human CG7734 homolog in mammalian (human) tissue. Quantitative analysis of human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1) expression in human abdominal adipocyte cells during the differentiation from preadipocytes to mature adipocytes

