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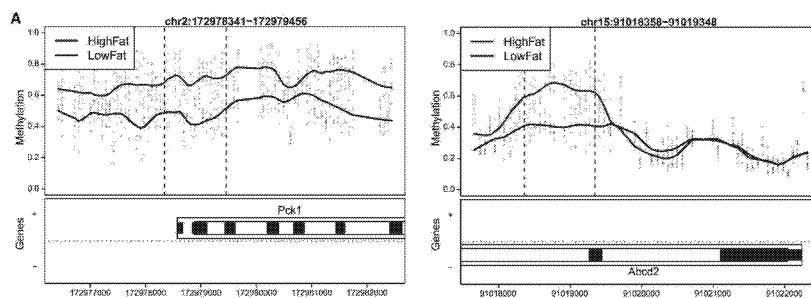
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(54) Title: METHOD OF EPIGENETIC ANALYSIS FOR DETERMINING CLINICAL GENETIC RISK



FIGS. 1A

(57) **Abstract:** The present invention provides a method for identifying a subject having or at risk of having a metabolic disease, such as diabetes or obesity. The invention is based on an approach to identify candidate genes involved in metabolic diseases, such as obesity and type 2 diabetes (T2D) through epigenetic mechanisms. The method includes identifying in the subject genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease. In another embodiment, the invention also provides a method of treating a subject having or at risk of having a metabolic disease. In another embodiment, the invention provides a method of providing a prognostic evaluation of a subject having or at risk of having a metabolic disease.

METHOD OF EPIGENETIC ANALYSIS FOR DETERMINING CLINICAL GENETIC RISK

RELATED APPLICATION DATA

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Serial No. 62/100,039, filed January 5, 2015, the entire contents of which is incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying sequence listing text file, name JHU3760_1WO_Sequence_Listing, was created on 04-January-2016, and is 30 kb. The file can be assessed using Microsoft Word on a computer that uses Windows OS.

STATEMENT OF GOVERNMENT SUPPORT

[0003] This invention was made in part with government support under Grant Nos. DP1 ES022579 and DK084171 awarded by the National Institutes of Health. The United States government has certain rights in this invention.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0004] The present invention relates generally to differentially methylated regions (DMRs) in the genome, and more specifically to methods for correlating DMRs with metabolic diseases or disorders.

BACKGROUND INFORMATION

[0005] The basis of modern disease association studies can be predicated on the "common disease common variant hypothesis," which argues that frequent variants in the general population, that arose at a point of historical population restriction, are associated with genetic variants for common disease. The concept is rooted in the neo-Darwinian synthesis of the previous century, and the population genetic analysis of R. A. Fisher, who argued that complex (multigenic) phenotypes arise additively from individual quantitative trait loci (QTLs). A great deal of effort has been expended on finding associations of common disease with single nucleotide polymorphisms (SNPs). While there have been important successes, the overwhelming majority of genome-wide associations studies (GWAS) have shown associations characterized by low odds ratios, around 70% report odd-ratio below 2, with generally relatively weak genome-wide statistical significance. This is a well-recognized

problem in the GWAS community, and has led to discussions of sources of the missing “dark matter” of heritability, reviewed recently in the literature. Alternatives include copy number variants, and rare variants, although copy numbers also appear to account for a relatively small attributable risk of disease, e.g. <1% in schizophrenia. A major goal of funding agencies is to extend sequencing efforts to much larger cohorts, and the identification of the major cause of disease-related genetic variation is essential to fulfill ambitions for personalized medicine, i.e., targeting therapy and disease risk mitigation based on one's genome.

[0006] A role for epigenetics in common disease has long been suspected, and a strong relationship with cancer has been shown. It is likely that common disease involves both genetic and epigenetic factors and that epigenetic modification could mark both environmental effects as well as mediate genetic effects. In addition to particular exposure-epigenetic relationships, epigenetic changes with aging support the notion that there is an environmental component to epigenetic variation. Studies of identical twins show greater differences in global DNA methylation in older than in younger twins, consistent with an age-dependent progression of epigenetic change. Global methylation changes over an 11 year span in participants of an Icelandic cohort, and age- and tissue-related alterations in some CpG islands from an array of 1,413 arbitrarily chosen CpG sites near gene promoters, further corroborate the evidence for dynamic methylation patterns over time. Other work, however, has suggested that epigenetic marks, or their maintenance, are themselves controlled by genes, and are thus heritable in the traditional sense and associated with particular DNA variants. This would predict that methylation marks are stable, rather than varying as controlled by changing environments.

[0007] A tenet of Origin of Species argues that phenotype is the result of many discrete traits that are individually and exquisitely selected, to quote Darwin, "detecting the smallest grain in the balance of fitness," which has been described as Newtonian in its dependence on static forces acting in consistent ways. This concept is the basis for quantitative trait loci that has been proposed in the scientific field. This concept has led to the modern basis of population genetics that continuous variation exists within a population, yet selection is on individuals, which has led to models of balancing or purifying selection at the extremes of phenotype. The classic model also has significant limitations in explaining common human disease; common variants can explain only a small fraction of a given disease phenotype, even the most well understood, such as adult-onset diabetes and height.

[0008] Epigenetics, the study of non-sequence-based changes in DNA and associated proteins, was first suggested to play a role in evolution through Lamarckian inheritance, that is, direct modification of the genome by the environment, which is then transmitted transgenerationally. Two examples are commonly cited: changes in coat color caused by dietary modifications of DNA methylation of the agouti gene in mice and methylation of the axin-fused allele in kinked tail mice. Both of these examples involve methylation of a retrotransposon LTR sequence, and thus fit into various genetic exceptions to classical Darwinian thinking, including anticipation due to trinucleotide repeat expansion and lateral gene transfer in the evolution of influenza strains. But they have not been shown to be general mechanisms for either speciation or developmental differences across species, so-called “evo-devo,” or for canalization, a term coined to refer to a mechanism by which environmental perturbations during development are corrected by the genetic program, leading to a consistent developmental plan.

[0009] Indeed, canalization remains a “black box,” as noted by some in the scientific field. Others have discussed the potential role for Lamarckian inheritance in disease; for example, some have proposed a model of transgenerational epigenetic Lamarckian inheritance and noted that such modifications must persist for many generations to contribute substantially to average risk, which has implications for public health management. Although not disputing an important contribution of Lamarckian inheritance, here the invention provides an alternative view in which genetic modification could provide stochastic phenotypic variation favored by selection in changing environments, and also provide an alternative non-Lamarckian role for epigenetics in evolution.

[0010] Thus, there is a need for a genome-scale analysis of DNA methylation to correlate epigenomics and clinical genetic risk.

SUMMARY OF THE INVENTION

[0011] The invention is based on an approach to identify candidate genes involved in metabolic diseases, such as obesity and type 2 diabetes T2D through epigenetic mechanisms. This approach may also be utilized to identify genes involved in numerous diseases in addition to metabolic diseases.

[0012] Accordingly, in one embodiment, the invention provides a method for identifying a subject having or at risk of having a metabolic disease. The method includes identifying in the subject genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the

markers with a control sample from a subject not having the disease. In one embodiment, the disease is T2D. The method of the invention further includes analyzing adipose cells of the subject, wherein an inflammatory response is a factor associated with having or risk of having a metabolic disease, such as T2D.

[0013] In another embodiment, the invention also provides a method of treating a subject having or at risk of having a metabolic disease. The method includes increasing or decreasing gene expression of a genetic marker identified by the method of the invention based on an observation of hypomethylation or hypermethylation, respectively, of the marker, thereby treating the subject. In one embodiment, the genetic marker affects glucose utilization by a cell. In another embodiment, the genetic marker(s) is associated with obesity. In another embodiment, the genetic marker is one or more markers set forth in Table 2.

[0014] In another embodiment, the invention provides a method of providing a prognostic evaluation of a subject having or at risk of having a metabolic disease. The method includes analyzing one or more of the subject's genetic markers identified in the method of the invention prior to dietary and/or pharmaceutical intervention and following dietary and/or pharmaceutical intervention, and correlating a change in the genetic markers with a prognostic evaluation of the subject. In one embodiment, a decrease in expression of a marker previously up-regulated is correlated with improvement in the disease. In another embodiment, an increase in expression of a marker previously down-regulated is correlated with improvement in the disease.

[0015] In yet another embodiment, the invention provides a method for identifying a subject having or at risk of having a disease, such as for example, a metabolic disease, cancer, immune system disorder, cardiovascular disease, gastrointestinal disease or pulmonary disease. The method includes identifying in the subject one or more genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease.

[0016] In another embodiment, the invention provides a method of determining a therapeutic regimen for a subject. The method includes identifying in the subject one or more genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject thereby assessing the therapeutic regimen for the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figures 1A-1B are graphical representations of data pertaining to genome-wide significant methylation changes related to diet-induced obesity in C57BL/6 mice.

[0018] Figures 2A-2B are graphical representations of data illustrating replication of mouse methylation changes in additional mice and associated gene expression changes.

[0019] Figures 3A-3B are graphical representations of data illustrating overlapping methylation changes in human and mouse adipose tissue.

[0020] Figures 4A-4B are diagrammatic representations of the interactions between epigenetically conserved and genetically associated genes implicated in this study.

[0021] Figure 5A-5C are graphical representation of data illustrating overexpression and shRNA-mediated knockdown of selected genes in 3T3-L1 adipocytes.

[0022] Figure 6 is a diagrammatic representation illustrating genetic characteristics of lean mice versus obese mice.

[0023] Figure 7 is a series of graphical representations of data representing correlation of metabolic traits in a diet-induced obesity mouse model, related to Figure 2.

[0024] Figures 8A-8B are graphical representations of data illustrating correlation of methylation and gene expression in mouse and human adipose tissue, related to Figure 2.

[0025] Figures 9A-9C are graphical representations of data illustrating significance of methylation change overlap between mouse and human tissues, related to Figure 3.

[0026] Figure 10 is a graphical representation of data illustrating enrichment of connections between genes implicated by methylation and genome-wide significant GWAS genes, related to Figure 4.

DETAILED DESCRIPTION OF THE INVENTION

[0027] Using a functional approach to investigate the epigenetics of metabolic diseases, such as T2D, the invention methods are based on a combination of three lines of evidence (diet-induced epigenetic dysregulation in mouse, epigenetic conservation in humans, and T2D clinical risk evidence) to identify genes implicated in T2D pathogenesis through epigenetic mechanisms related to obesity. Beginning with dietary manipulation of genetically homogeneous mice, differentially DNA-methylated genomic regions were identified. These results were then replicated in adipose samples from lean and obese patients pre- and post-Roux-en-Y gastric bypass, identifying regions where both the location and direction of methylation change is conserved. These regions overlap with 27 genetic T2D risk loci, only one of which was deemed significant by GWAS alone. Functional analysis of genes

associated with these regions revealed four genes with roles in insulin resistance, demonstrating the potential general utility of this approach for complementing conventional human genetic studies by integrating cross-species epigenomics and clinical genetic risk. While diabetes is provided as an illustrative example, it is believed that the analyses provided herein are applicable to epigenomics and clinical genetic risk for other metabolic diseases as well as cancer, immune system disorder, cardiovascular disease, gastrointestinal disease or pulmonary disease.

[0028] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0029] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning 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 invention, the preferred methods and materials are now described.

[0031] The present invention establishes an approach utilizing two species to identify candidate genes involved in obesity and T2D through epigenetic mechanisms. The experiments described herein examined the epigenetic consequences of a high-fat diet in a carefully controlled experimental mouse obesity setting. They then replicated across species-in humans-by analyzing adipose tissue from a cohort that both reproduces and reverses a phenotype similar to the obese mouse. The use of samples from the same subjects pre- and post-RYGB allows a human isogenic comparison of the effect of obesity-induced metabolic disturbances. This cross-species approach exploits the power of evolutionary selection, whose mechanisms have survived the 50 million year separation between mouse and human, in a more comprehensive manner than simple replication from human set to human set, and may better identify functionally important environmental targets. They lastly stratified these cross-

species obesity-associated regions using genetic association data from a large genome-wide association study (GWAS) for T2D to more directly link the obesity-derived phenotypes with human T2D. As a result of this approach, the invention provides a method to identify genes with roles in insulin resistance, suggesting that this cross-species approach provides a powerful experimental system for identifying the genomic variation associated with common disease.

[0032] Accordingly, in one embodiment, the invention provides a method for identifying a subject having or at risk of having a metabolic disease. The method includes identifying in the subject genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease.

[0033] A metabolic disease as used herein includes diseases that affect glucose utilization by a cell. Such diseases may include obesity, pre-diabetes, diabetes and the like. As illustrated in the Examples, the metabolic disease may be T2D. While the invention has identified genetic markers which are associated with metabolic disease, and in particular, obesity and diabetes, it will be understood by one in the art, the a similar approach may be taken to identify genetic markers associated with other types of diseases, for example, cancer, immune system disorder, cardiovascular disease, gastrointestinal disease and pulmonary disease.

[0034] As used herein, a “genetic marker” refers to, a nucleic acid molecule, such as a gene, gene promoter, or other region of a genome that may be observed and correlated with a disease. For example, a genetic marker may refer to a gene or other portion of a genome which may be assessed for methylation status. In this manner, a genetic marker includes a gene or differentially methylated region (DMR) of a genome. In various embodiments of the present invention, a genetic marker includes one or more genes or DMRs associated with one or more genes set forth in Table 2. For example, the genetic marker may be one or more genes or DMRs associated with Tcf7l2, As3mt, Etaa1, Tnfsf8, Plekho1, Tnfaip8l2, Akt2, Lhfpl2, Mkl1, BC048644 (Car5a), Rgs3, Fgd3, Stau1, Tmcc3, Tbx3, Gatz1, Taok3, Bnip3, Dlst, Kcna3, Cln8, Cd37, Nfib, Pck1, Pcx, Hoxd3, Cd33 or Evl. In a particular embodiment, the genetic marker includes at least Tcf7l2, or one or more of Mkl1, Plekho1 and Tnfaip8l2. For example, the genetic marker may include Tcf7l2 alone, Tcf7l2 in combination with one or more of Mkl1, Plekho1 and Tnfaip8l2, or Tcf7l2 in combination with one or more of Tcf7l2, As3mt, Etaa1, Tnfsf8, Plekho1, Tnfaip8l2, Akt2, Lhfpl2, Mkl1, BC048644 (Car5a),

Rgs3, Fgd3, Stau1, Tmcc3, Tbx3, Gatz1, Taok3, Bnip3, Dlst, Kcna3, Cln8, Cd37, Nfib, Pck1, Pcx, Hoxd3, Cd33 or Evl.

[0035] In another embodiment, the invention also provides a method of treating a subject having or at risk of having a metabolic disease. The method includes increasing or decreasing gene expression of a genetic marker identified by the method of the invention based on an observation of hypomethylation or hypermethylation, respectively, of the marker, thereby treating the subject.

[0036] Gene expression in the subject may be altered using various techniques as known in the art. For example, gene expression may be increased or decreased by administering an agent to the subject that effects gene expression. An agent, as used herein, is intended to include any agent capable of altering gene expression, for example, by altering the methylation status of a nucleic acid molecule. For example, an agent useful in any of the methods of the invention may be any type of molecule, for example, a polynucleotide, a peptide, a peptidomimetic, peptoids such as vinyllogous peptoids, chemical compounds, such as organic molecules or small organic molecules, or the like. In various aspects, the agent may be a polynucleotide, such as DNA molecule, an antisense oligonucleotide or RNA molecule, such as microRNA, dsRNA, siRNA, stRNA, and shRNA.

[0037] In another embodiment, the invention provides a method of providing a prognostic evaluation of a subject having or at risk of having a metabolic disease. The method includes analyzing one or more of the subject's genetic markers identified in the method of the invention prior to dietary and/or pharmaceutical intervention and following dietary and/or pharmaceutical intervention, and correlating a change in the genetic markers with a prognostic evaluation of the subject. In one embodiment, a decrease in expression of a marker previously up-regulated is correlated with improvement in the disease. In another embodiment, an increase in expression of a marker previously down-regulated is correlated with improvement in the disease.

[0038] In yet another embodiment, the invention provides a method for identifying a subject having or at risk of having a disease, such as, a metabolic disease, cancer, immune system disorder, cardiovascular disease, gastrointestinal disease or pulmonary disease. The method includes identifying in the subject one or more genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease.

[0039] In another embodiment, the invention provides a method of determining a therapeutic regimen for a subject. The method includes identifying in the subject one or more genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject thereby assessing the therapeutic regimen for the subject.

[0040] In the present invention, the subject is typically a human but also can be also be any non-human mammal or other classes, including, but not limited to, a dog, cat, rabbit, cow, bird, rat, horse, pig, or monkey.

[0041] In the various methods of the invention, methylation status of a nucleic acid molecule, such as a gene, or a region of a genome identified as a DMR and correlated with a disease is assessed. In various aspects of the invention a genetic marker such as a gene or DMR may be hypermethylated or hypomethylated as compared to a control. Hypomethylation is present when there is a measurable decrease in methylation. In some embodiments, a marker can be determined to be hypomethylated when less than 50% of the methylation sites analyzed are not methylated. Hypermethylation is present when there is a measurable increase in methylation. In some embodiments, a marker can be determined to be hypermethylated when more than 50% of the methylation sites analyzed are methylated. Methods for determining methylation states are provided herein and are known in the art. In some embodiments methylation status is converted to an M value. As used herein an M value, can be a log ratio of intensities from total (Cy3) and McrBC-fractionated DNA (Cy5): positive and negative M values are quantitatively associated with methylated and unmethylated sites, respectively. M values are calculated as described in the Examples. In some embodiments, M values which range from -0.5 to 0.5 represent unmethylated sites as defined by the control probes, and values from 0.5 to 1.5 represent baseline levels of methylation.

[0042] Numerous methods for analyzing methylation status of a gene are known in the art and can be used in the methods of the present invention to identify either hypomethylation or hypermethylation. In some embodiments, bisulfite pyrosequencing, which is a sequencing-based analysis of DNA methylation that quantitatively measures multiple, consecutive CpG sites individually with high accuracy and reproducibility, may be used. Exemplary primers for such analysis are set forth in Tables 3 and 4.

[0043] It will be recognized that depending on the site bound by the primer and the direction of extension from a primer, that the primers listed above can be used in different

pairs. Furthermore, it will be recognized that additional primers can be identified within the DMRs, especially primers that allow analysis of the same methylation sites as those analyzed with primers that correspond to the primers disclosed herein.

[0044] Altered methylation can be identified by identifying a detectable difference in methylation. For example, hypomethylation can be determined by identifying whether after bisulfite treatment a uracil or a cytosine is present a particular location. If uracil is present after bisulfite treatment, then the residue is unmethylated. Hypomethylation is present when there is a measurable decrease in methylation.

[0045] In an alternative embodiment, the method for analyzing methylation can include amplification using a primer pair specific for methylated residues within a nucleic acid molecule. In these embodiments, selective hybridization or binding of at least one of the primers is dependent on the methylation state of the target DNA sequence (Herman et al., *Proc. Natl. Acad. Sci. USA*, 93:9821 (1996)). For example, the amplification reaction can be preceded by bisulfite treatment, and the primers can selectively hybridize to target sequences in a manner that is dependent on bisulfite treatment. For example, one primer can selectively bind to a target sequence only when one or more base of the target sequence is altered by bisulfite treatment, thereby being specific for a methylated target sequence.

[0046] Other methods are known in the art for determining methylation status, including, but not limited to, array-based methylation analysis and Southern blot analysis.

[0047] Methods using an amplification reaction, for example methods above for detecting hypomethylation or hypermethylation of one or more DMRs, can utilize a real-time detection amplification procedure. For example, the method can utilize molecular beacon technology (Tyagi et al., *Nature Biotechnology*, 14: 303 (1996)) or Taqman™ technology (Holland et al., *Proc. Natl. Acad. Sci. USA*, 88:7276 (1991)).

[0048] Also methyl light (Trinh et al., *Methods* 25(4):456-62 (2001), incorporated herein in its entirety by reference), Methyl Heavy (Epigenomics, Berlin, Germany), or SNUPE (single nucleotide primer extension) (see e.g., Watson et al., *Genet Res.* 75(3):269-74 (2000)) Can be used in the methods of the present invention related to identifying altered methylation of DMRs.

[0049] The degree of methylation in the DNA associated with the DMRs being assessed, may be measured by fluorescent *in situ* hybridization (FISH) by means of probes which identify and differentiate between genomic DNAs, associated with the DMRs being assessed, which exhibit different degrees of DNA methylation. FISH is described, for example, in de

Capoa et al. (*Cytometry*. 31:85-92 (1998)) which is incorporated herein by reference. In this case, the biological sample will typically be any which contains sufficient whole cells or nuclei to perform short term culture. Usually, the sample will be a sample that contains 10 to 10,000, or, for example, 100 to 10,000, whole cells.

[0050] Additionally, as mentioned above, methyl light, methyl heavy, and array-based methylation analysis can be performed, by using bisulfite treated DNA that is then PCR-amplified, against microarrays of oligonucleotide target sequences with the various forms corresponding to unmethylated and methylated DNA.

[0051] To examine DNAm on a genome-wide scale, comprehensive high-throughput array-based relative methylation (CHARM) analysis, which is a microarray-based method agnostic to preconceptions about DNAm, including location relative to genes and CpG content may be utilized. The resulting quantitative measurements of DNAm, denoted with *M*, are log ratios of intensities from total (Cy3) and McrBC-fractionated DNA (Cy5): positive and negative *M* values are quantitatively associated with methylated and unmethylated sites, respectively. For each sample, ~4.6 million CpG sites across the genome of a may be analyzed. In embodiments, methylation status is determined according to the method set forth in Irizarry et al. (*Genome Res.* 18:780-790 (2008)) or Ladd-Acosta et al. (*Current Protocols in Human Genetics* 20.1.1-20.1.19 (2010)), both of which are incorporated herein by reference in their entireties.

[0052] In various embodiments, the determining of methylation status in the methods of the invention is performed by one or more techniques selected from the group consisting of a nucleic acid amplification, polymerase chain reaction (PCR), methylation specific PCR, bisulfite pyrosequencing, single-strand conformation polymorphism (SSCP) analysis, restriction analysis, microarray technology, and proteomics. As illustrated in the Examples herein, analysis of methylation can be performed by bisulfite genomic sequencing. Bisulfite treatment modifies DNA converting unmethylated, but not methylated, cytosines to uracil. Bisulfite treatment can be carried out using the METHYLEASYTM bisulfite modification kit (Human Genetic Signatures).

[0053] In the various methods of the invention, genetic markers can be identified from a sample from the subject. A sample can be taken from any tissue that is susceptible to disease. A sample may be obtained by surgery, biopsy, swab, stool, or other collection method. In some embodiments, the sample is derived from blood, adipose tissue, pancreatic tissue, liver

tissue, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, stool, skin, hair or tears.

[0054] The following examples are provided to further illustrate the advantages and features of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE I

MOUSE-HUMAN EXPERIMENTAL EPIGENETIC ANALYSIS UNMASKS DIETARY TARGETS AND GENETIC LIABILITY FOR DIABETIC PHENOTYPES

[0055] The inventors established an approach utilizing two species to identify candidate genes involved in obesity and Type 2 Diabetes (T2D) through epigenetic mechanisms. The inventors first examined the epigenetic consequences of a high-fat diet in a carefully controlled experimental mouse obesity setting. The inventors then replicated across species (in humans) by analyzing adipose tissue from a cohort that both reproduces and reverses a phenotype similar to the obese mouse. The use of samples from the same subjects pre- and post-RYGB allows a human isogenic comparison of the effect of obesity-induced metabolic disturbances. This cross-species approach exploits the power of evolutionary selection, whose mechanisms have survived the 50 million year separation between mouse and human, in a more comprehensive manner than simple replication from human set to human set, and may better identify functionally important environmental targets. The inventors lastly stratified these cross-species obesity-associated regions using genetic association data from a large genome-wide association study (GWAS) for T2D to more directly link the obesity-derived phenotypes with human T2D. As a result of this approach, the inventors are able to identify four genes with roles in insulin resistance, suggesting that this cross-species approach provides a powerful experimental system for identifying the genomic variation associated with common disease.

[0056] The following experimental protocols and materials were utilized.

[0057] Mouse Sample Preparation

[0058] All animal protocols were approved by the Institutional Animal Care and Use Committee of The Johns Hopkins University School of Medicine. Male C57BL/6 mice were purchased from Charles River and housed in polycarbonate cages on a 12-h light-dark photocycle with ad libitum access to water and food. Mice were fed a high-fat diet (HFD; 60% kcal derived from fat, Research Diets; D12492) or the matched control low-fat diet

(LFD; 10% kcal derived from fat, Research Diets; D12450B). Diet was provided for a period of 12 weeks, beginning at 4 weeks of age. At termination of the study, animals were fasted overnight and euthanized; tissues were collected, snap frozen in liquid nitrogen, and kept at -80°C until analysis.

[0059] Intraperitoneal Glucose and Insulin Tolerance Tests

[0060] Cohorts of mice (between 20 and 24 weeks of age) were injected with glucose (1 g/kg body weight) or insulin (0.8 units/kg for LFD-fed mice, 1.2 units/kg for HFD-fed mice). Animals were fasted overnight (16 h) prior to the glucose tolerance test. For the insulin tolerance test, food was removed 2 h prior to insulin injection. Serum samples were collected by using microvette CB 300TM (Sarstedt). Glucose concentrations were determined at time of blood collection with a glucometer (BD Biosciences). Six blood samples were collected at sequential timepoints after injections.

[0061] Mouse Hepatocyte Isolation

[0062] A protocol for primary hepatocyte isolation was adapted from previously published methods. Mice were anesthetized and a catheter was inserted into the vena cava. The portal vein was then cut to allow liver-specific perfusion. Mice were then perfused with PBS, followed by 100ug/mL Type I Collagenase (BD Biosciences) at a rate of 5 ml/min for 10 min. The liver was then removed and dissociated by straining through a 70 μm pore nylon cell strainer (BD Falcon). The cells were then spun down and resuspended in William's Medium ETM (Cellgro). Primary hepatocytes were then isolated by gradient distribution via centrifugation of the resuspension in a cold PercollTM (GE healthcare) solution. Verification of primary hepatocyte purity was assessed via quantitative real-time PCR for hepatocyte-specific genes compared to markers for endothelial and immune cells. The inventors observed >90% hepatocyte purity based on gene expression.

[0063] Mouse Primary Adipocyte Isolation

[0064] Mature adipocytes were isolated from mouse fat pads as previously described. Briefly, fat pads were finely chopped using scissors. Tissue was then dissociated in 2 mg/gram tissue Type II Collagenase (Sigma) in KRH buffer. The digestion was stopped by adding 10% FBS (Atlantic Biologicals) to the mixture and cells were filtered through 100 μm pore nylon cell strainers (BD Falcon). The cells were then separated out by transferring the upper phase of cells to a new tube and washing with 5 mL of KR Buffer. The wash and resuspension was repeated 3 times and mature adipocytes were collected. Verification of mature adipocyte purity was assessed via quantitative real-time PCR for adipose-specific

genes compared to markers for endothelial and immune cells. The inventors observed >95% adipocyte purity based on gene expression.

[0065] Pancreatic Islet Isolation

[0066] Pancreatic islets used for CHARM were isolated as previously described. For the pancreatic islets used in the replication set, whole pancreases were obtained from high-fat-fed and lowfat-fed mice, stained for insulin using the Anti-Insulin + Proinsulin antibody [D3E7]TM (Biotin) (ab20756) (Abcam, MA, USA) kit, cryosectioned into 8µm sections, and then laser-capture microdissection was used to isolate pancreatic islets (PALM Microbeam, Carl Zeiss, NC, USA).

[0067] 3T3-L1 Transduction and Transfection

[0068] 3T3-L1 cells were transduced with Sigma MissionTM lentiviral particles and transfected with overexpression plasmids using LipofectamineTM 3000 (Life Technologies) as per the respective manufacturers' protocols. Cells were plated at 60% confluency and incubated for 18 hours in a humidified incubator. Media was removed and replaced by Opti-MEMTM (Invitrogen) with 8µg/ml Hexadimethrine Bromide (Sigma-Aldrich). Fifteen µl lentiviral particles were added and the plates were incubated for 18 hours in a humidified incubator. Media was then removed and replaced, and on the following day media containing 10µg/ml puromycin (Sigma Aldrich) was added and the cells were cultured in puromycin thereafter.

[0069] 3T3-L1 cells were transfected with overexpression plasmids using LipofectamineTM 3000 (Life Technologies) as per the manufacturer's protocol. Cells were plated at 60% confluency and incubated for 18 hours in a humidified incubator. LipofectamineTM 3000 (1.5µl per well containing cells) was diluted and mixed in 50µl Opti-MEM medium (Invitrogen). At the same time, 4µg plasmid DNA was diluted in 50µl Opti-MEM with 2µl P3000TM reagent and mixed. The diluted LipofectamineTM and plasmid DNA were then mixed, incubated for 5 min at room temperature, and distributed onto the plated cells. After 24 hours incubation, the media was replaced with growth media. After 48 hours, 500µg/ml Geneticin Selective AntibioticTM (G418 Sulfate, Life Technologies) was added, and the cells were maintained in geneticin thereafter.

[0070] Lentiviral particles used: Tmcc3 (TRCN0000126784, Sigma Aldrich), Gatz1 (TRCN0000103080, Sigma Aldrich), MISSION® TRC2 pLKO.5-puro Non-Mammalian shRNA Control Transduction ParticlesTM (Control, SHC202V, Sigma Aldrich).

[0071] Overexpression plasmids used: Mkl1 (MC202660, Origene), Plekho1 (MC210507, Origene), Tnfaip8l2 (MC203559, Origene), Cloning vector PCMV6-Kan/Neo (Control, PCMV6KN, Origene).

[0072] Cell Culture and Glucose Uptake Assay

[0073] 3T3-L1 cell lines (ATCC) were maintained in Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 10% FBS (Invitrogen), and 10 µg/ml puromycin and 500 µg/ml geneticin (G418) as selective antibiotics for the knock-down and overexpression lines, respectively. Two days after confluence, differentiation of the knock-down lines was induced by incubation with MDI medium (4 µg/ml insulin, 0.5mM Methylisobutylxanthine (IBMX), 1.0 µM dexamethasone) for 2 days and 4 µg/ml insulin for 5 days. Differentiation of the over-expression lines was induced with MDI medium and 1 µM rosiglitazone for 3 days and 4 µg/ml insulin for 3 days. After another 3-5 days of incubation with maintenance medium, 80%-100% differentiation was shown by lipid droplet accumulation in the cells. Glucose uptake assays were performed on differentiated knock-down and over-expression lines. After 2 h of incubation in serum-free DMEM, they were washed twice in pre-warmed PBS and placed in HEPES buffered saline solution (25 mM HEPES, pH 7.4, 120 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂, 1.3 mM KH₂PO₄, and 0.5% BSA) containing 10 nM or 100 nM insulin for 20 min. Then, 0.5 µCi/well 2-deoxy-D-[3H]glucose (Moravek) was added for 5 min. The reactions were terminated by two ice-cold PBS washes. Cells were then incubated for 10 min with whole cell lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, and 10% glycerol). The lysates were transferred to scintillation vials containing EcoscintTM scintillation fluid (National Diagnostics) and counted with a Beckman Coulter counter (model LS 6000SC).

[0074] Human Sample Surgery and Subcutaneous Adipose Tissue Biopsies

[0075] A standard laparoscopic RYGB with a 1 m Roux limb was performed. The patients were weight stable and not subjected to a preoperative weight loss period. Subcutaneous abdominal adipose biopsies (50– 100 mg) were obtained from the obese and non-obese (normal weight) subjects. Biopsies were obtained at the beginning of RYGB surgery (obese subjects) or elective laparoscopic cholecystectomy (lean subjects) after the induction of general anesthesia. Only non-glucose-containing intravenous solutions were administered before the biopsy was taken during RYGB or elective cholecystectomy surgery after an overnight fast. Biopsies taken from the obese subjects 6 months after RYGB surgery were obtained under local anesthesia (5 mg/ml of lidocaine hydrochloride) in the morning after an

overnight 12 hour fast from the same surgical incision as the initial biopsy. Biopsy samples for DNA analysis were immediately frozen and stored in liquid nitrogen until analysis. Fat and liver biopsies were obtained at the beginning of RYGB surgery (obese subjects) or elective laparoscopic cholecystectomy (lean subjects) after the induction of general anesthesia.

[0076] CHARM DNA Methylation Analysis

[0077] Genomic DNA from all samples was purified with the MasterPure™ DNA purification kit (Epicentre) following the manufacturer's protocol. Genomic DNA (1.5–2 µg) was fractionated with a Hydroshear Plus™ (Digilab), digested with McrBC, gel-purified, labeled and hybridized to a CHARM microarray as described. The mouse CHARM 2.0™ array used in the analysis now includes 2.1 million probes, which cover 5.2 million CpGs arranged into probe groups (where consecutive probes are within 300 bp of each other) that tile regions of at least moderate CpG density. The human CHARM 3.0™ array now includes 4.1 million probes, which cover 7.5 million CpGs. These arrays include all annotated and non-annotated promoters and microRNA sites on top of the features that are present in the original CHARM method. The inventors dropped 7 human arrays with <80% of their probes above background intensities, resulting in 11 pre-surgery obese samples, 8 post-surgery obese samples, and 8 lean samples that underwent DNA methylation analysis. The design specifications are freely available on the World Wide Web at rafalab.jhu.edu. The inventors then removed sex chromosomes to improve the batch correction methods.

[0078] Subsequent pre-processing, normalization and correction for batch effects were performed as previously described. Briefly, the inventors applied a “bump hunting” approach which involves a) performing linear regression at each probe, comparing DNA methylation levels versus a covariate of interest (e.g. high- versus low-fat diet), adjusting for surrogate variables, b) smoothing the regression coefficient for the covariate of interest across nearby probes and c) thresholding these smoothed regression coefficients across all probe groups, which forms differentially methylated regions (DMRs) representing adjacent probes with statistics above the threshold. Each DMR is summarized by its “area”, or the sum of the adjacent statistics above the threshold. The inventors used the 99.9th percentile of the smoothed statistics for each respective species, tissue and trait comparisons bump hunting analysis. Statistical significance was assessed via linear model bootstrapping, retaining surrogate variables, followed by bump hunting, which approximates full permutation (e.g.

permuting trait, recalculating surrogate variables, then bump hunting) using much less computational time.

[0079] Bisulfite Pyrosequencing

[0080] Genomic DNA (gDNA, 200 ng) from each replication sample was bisulfite treated using the EZ DNA Methylation-Gold™ Kit (Zymo research) according to the manufacturer's protocol. Bisulfite-treated gDNA was PCR amplified using nested primers, and DNA methylation was subsequently determined by pyrosequencing with a PSQ HS96 (Biotage) as previously reported. Artificially methylated control standards of 0, 25, 50, 75 and 100% methylated samples were created using mixtures of purified and SssI-treated whole genome amplified (REPLI-g™ amplification kit, Qiagen) Human Genomic DNA: Male™ (Promega). Pyrosequencing primers are shown in Table 3.

[0081] Quantitative PCR Analysis

[0082] Validated primers for all genes were taken from PrimerBank™ and synthesized by Integrated DNA Technologies (Coralville, IA, USA). RNA was extracted with Trizol reagent (Life Technologies, Carlsbad, CA, USA), cDNA was created with Quantitect Reverse Transcriptase Kit™ (Qiagen, Venlo, Netherlands), and quantitative-PCR was performed with Fast SYBR Green™ (Applied Biosystems, Foster City, CA, USA) on a 7900HT Fast Real-Time PCR™ system (Applied Biosystems, Foster City, CA, USA). RNA levels were normalized to same-sample 18S RNA levels. Quantitative PCR primers are shown in Table 4.

[0083] GO Annotation

[0084] The inventors analyzed GO annotation using the GOrilla™ tool. Enrichment was calculated by comparing genes identified from the analysis to a background of all genes detectable on the appropriate array.

[0085] Whole-Genome Gene Expression Analysis

[0086] Whole genome gene expression data for mouse and human analogues of the study was downloaded from GEO. The mouse data was already pre-processed, and the human data was pre-processed using Robust Multi-array Averaging™ (RMA) from the Affy R™ library (Bioconductor). The gene expression data was then matched against the DMRs closest to corresponding genes, the log fold change (logFC) of the gene expression was plotted against the average value of the smoothed effect estimate within the DMR, and p-values were generated using t-tests based on Pearson's correlation coefficient.

[0087] Enrichment Between Human and Mouse DMRs

[0088] The liftOver[™] tool from the UCSC genome browser transformed the coordinates from the human DMRs from the hg19 human genome to the mm9 mouse genome, as implemented in the *rtracklayer* Bioconductor[™] package. The locations of the 249,094 probe groups on the human CHARM array were also lifted over to serve as the natural background for enrichment, of which 214,646 (86.2%) had any analogous sequence in mouse, and a further 109,234 (50.9%) were within 5kb of a mouse CHARM probe group. For each pair of DMR lists, one from the two lifted-over human DMRs and another from the 25 mouse trait DMRs (see Table S1 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015)) publicly available on the World Wide Web at sciencedirect.com/science/article/pii/S1550413114005658, which is incorporated herein by reference in its entirety; Table S1 shows the results of CHARM analysis for five assayed mouse tissues against five measured metabolic phenotypes of diet, fasting glucose, mouse weight, glucose tolerance test and insulin tolerance test and is related to Table 1 herein), the inventors calculated the number of DMRs at given within specific p-value significance levels, and also the number that overlapped within 5kb across species. Enrichment tests were chi-squared tests based on the number of species-overlapping significant DMRs, then DMRs only significant within each species, and finally the number of lifted probe group (of the 109,234) that were not significant in either species (which creates a 2x2 table of the number significant in both species, significant in just human, significant in just mouse, and significant in neither species). This is analogous to creating a Venn diagram between significant human and mouse DMRs.

[0089] Cross-species Statistical Analysis

[0090] The inventors combined significant adipocyte mouse DMRs (at FDR < 5%) across the five traits (glucose, GTT, ITT, weight, and diet) by retaining the maximal coordinates over overlapping cross-trait DMRs resulting in 625 independent DMRs associated with at least 1 trait in adipocytes in mouse. These regions were lifted over from the mouse mm9 genome build to the human hg19 genome build as implemented in the *rtracklayer* Bioconductor package (Lawrence et al., 2009). These DMRs were annotated to the nearest human charm probe group based on the annotation within 5kb. The inventors then computed a difference and corresponding p-value in obese versus lean and then in obese humans pre-versus post RYGB surgery using linear regression, and retained the minimum p-value, number of probes with $p < 0.05$, and the slope at the smallest p-value, within each of the mapped DMRs.

[0091] DIAGRAM GWAS Analysis

[0092] The inventors integrated GWAS results into the 497 mouse-human DMRs by obtaining publicly available results from the DIAGRAM meta-analysis (available on the World Wide Web at diagram-consortium.org/downloads.html; Stage 1 GWAS: Summary Statistics download) with coordinates in genome build hg18. The separate GWAS studies that make up this meta-analysis have each been corrected for population structure differences, and the meta-analysis summary statistics (e.g. test statistics and p-values per SNP) are available for public download. The inventors then generated regions of high genotypic correlation by taking all SNP rs numbers with $p < 0.01$ ($n=39,081$) passing them through the SNAP tool using CEU 1000 Genomes Pilot 1 data (Johnson et al., 2008), obtaining proxy SNPs with $R^2 > 0.8$ ($n=167,055$ unique proxies), and recording the coordinate range of the proxies for each SNP. Overlapping per-SNP risk regions were merged if overlapping ($n=7,946$ genotypic risk regions) and the smallest p-value across all merged SNPs represented the p-value for the genotypic risk region. These genotypic regions were lifted over to hg19 coordinates for cross-species analysis as described above. The inventors estimated the variance in disease susceptibility based on disclosed algorithms using 1000 genomes-derived risk allele frequencies and assuming a disease prevalence of 8% for a given collection of risk SNPs.

[0093] The inventors assessed potential enrichment between DMRs and the GWAS results using two complementary approaches. The first approach assessed the enrichment in genome location between DMRs and the LD blocks from the GWAS. This permutation-based enrichment test is performed on two lists of genomic regions (e.g. chr:start-end) that assesses the degree of overlap relative to the background genome. At a given GWAS p-value cutoff, the inventors counted the proportion of GWAS signals that overlapped at least 1 DMR, and then generated background overlap by resampling the same number of GWAS regions (and the same length distribution) 10,000 times from the mappable genome (e.g. the genome after removing coordinates corresponding to telomeres, centromeres and other gaps present in genome build hg19, available from UCSC). Empirical p-values for enrichment were calculated by counting the number of null proportions that were greater than the observed proportion. R code is available on GitHubTM.

[0094] The second approach assessed enrichment in gene symbols based on all genes directly connected (one-step) to genes linked to T2D with genome-wide significance by the DIAGRAM meta-analysis based on regulatory networks generated using Qiagen's Ingenuity IPATM. These sets (also known as interaction networks in Ingenuity) were able to be

generated for 57 out of 59 genome-wide significant genes. Full interaction networks were not able to be retrieved for the remaining two genes, and these were excluded from the analysis. These interaction networks then had chemicals, groups, complexes and miRNAs filtered in order to limit the potential interacting partners to genes and protein products.

[0095] The inventors computed whether genes overlapping obesity-related DMRs were more likely to be associated with GWAS genes and their interaction networks. The inventors first removed DMRs that were not within 10kb of a RefSeq gene, leaving 244 and 471 obesity-related DMRs in islet and adipose tissue respectively (from 312 and 576). Then the inventors counted the number of GWAS-associated genes and their directly connected partners in the genes containing DMRs. This procedure was also performed after the cross-species conservation filtering step described above, leaving 44 and 146 conserved obesity-related DMRs overlapping genes. The inventors obtained statistical significance based on a resampling analysis, where the inventors resampled the same number of probes groups 100,000 times from all probes groups mapped to human genes on the mouse CHARM design by: 1) lifting the range of the coordinates of each probe group to hg19, 2) removing poorly lifted probes groups defined as greater than 1.5 times the longest (in bp) original probe group prior to lifting over, 3) assigning the nearest human gene to each lifted probe group, and 4) dropping lifted probes groups not within 10kb of a human RefSeq gene. The inventors counted the number of GWAS signals or their directly connected partners that overlapped the resampled genes in each iteration, and calculated an empirical p-value based on this null distribution. This procedure was therefore performed four times, for both adipose and islet DMRs with and without filtering for cross-species conservation.

[0096] Data Availability

[0097] Both raw and processed microarray data has been uploaded to GEO, the Gene Expression OmnibusTM, as series record GSE63981.

[0098] Results

[0099] Alterations in DNA Methylation in Mouse Adipocytes Produced by High-Fat Diet

[0100] To detect DNA methylation differences, the inventors used the comprehensive high-throughput array-based relative methylation (CHARM) method, which in its current form can assay over 5 million CpG sites in mouse and 7.5 million CpG sites in human. In 12 adipocyte samples extracted from mouse adipose tissue, the inventors found 232

differentially methylated regions (DMRs) correlated with diet status (Table 1). As an example, when comparing adipocytes from high-fat-fed mice versus low-fat-fed mice, the inventors found hypermethylation overlying the promoter of phosphoenolpyruvate carboxykinase 1 (*Pck1*, Figure 1A). PEPCCK, the product of *Pck1*, catalyzes a rate-limiting step in gluconeogenesis, is essential for lipid metabolism in adipose tissue, is known to be regulated by insulin, and has been linked to lipodystrophy and obesity in mice.

[0101] Figures 1A-1B are graphical representations of data illustrating genome-wide significant methylation changes related to diet-induced obesity in C57BL/6 mice. In Figure 1A, two genome-wide significant DMRs are hypermethylated in adipocytes purified from mice raised on a high-fat diet. Each point represents the methylation level in adipocytes from an individual mouse at a specific probe, with smoothed lines representing group methylation averages. These points are colored blue for lean mice and red for obese mice.

[0102] In Figure 1B, body weight (grams) and glucose tolerance (AUC) are associated with methylation in adipocytes at genome-wide significant levels. Each point in the top panels represents one probe, with the y axis representing the Pearson correlation coefficients of the probes with the analyzed phenotype. Dotted lines represent the extent of the DMR as generated automatically via CHARM. The bottom panels display gene location information for the chromosomal coordinates on the x axis.

[0103] In addition to the high-fat versus low-fat analysis, even more DMRs were detected when analyzing methylation differences related to the metabolic phenotypes of body weight, fasting glucose, and insulin and glucose tolerance test area-under-curve (ITT/GTT AUC) values (see Table 1 herein and Table S1 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015))). One example of a mouse GTT-associated DMR is in the *Fasn* gene, which produces fatty acid synthase. Most DMRs found were significantly associated with more than one trait, which is not entirely unexpected as the phenotypes themselves are highly correlated (Figure 7).

[0104] Figure 7 is a series of graphical representations of data representing correlation of metabolic traits in a diet-induced obesity mouse model, related to Figure 2. The Figure shows correlations between the mouse traits observed over time. Mouse weight, fasting glucose levels (collected at the time of glucose tolerance test), and insulin tolerance test and

glucose tolerance test area-under-the-curve scores are plotted and correlated against each other. Correlation coefficients and p-values for the linear models are shown in the inserts.

[0105] The inventors additionally examined DNA methylation in pancreatic islets purified from whole mouse pancreata and hepatocytes extracted from mouse liver tissue. The inventors found significant correlations between methylation and mouse diet and weight in pancreatic islets and correlations between methylation and weight and ITT in hepatocytes (see Table S1 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015))).

[0106] Pooling tissues together and surveying for DNA methylation changes in common across tissues yielded no significant results.

[0107] Gene Ontology for Mouse DMRs

[0108] The inventors implemented gene set analyses to assess the overall biological importance of the DNA methylation changes the inventors observed in mouse adipocytes. The genome-wide significant adipocyte DMRs were near genes that were significantly overrepresented in lipid metabolic and immune/inflammatory pathways compared to the background list of genes represented on the array, with enrichment q values $< 9.7 \times 10^{-3}$ (Table 5). Examining hyper- and hypomethylated DMRs separately in high-fat-fed obese mice, the inventors observed that the metabolic pathway enrichment was derived from genes near hypermethylated DMRs, while the inflammatory pathway enrichment was present mainly in genes near hypomethylated DMRs.

[0109] Inflammatory and immune-related systems are known to be upregulated in adipocytes specifically in both obesity and T2D. Similarly, recent work has shown adipose de novo lipogenesis downregulation associated with metabolic dysfunction. These pathways, however, have not previously been shown to be significantly associated with methylation changes in a diet-induced obesity phenotype.

[0110] Methylation Replication in Mice and Associated Gene Expression Studies

[0111] The inventors then tested for replication of the methylation results at nine DMRs in adipocytes and three DMRs in pancreatic islets in an independent set of 18 mice (see Figure 2A herein and Table S3 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015))) publicly available on the World Wide Web at sciencedirect.com/science/article/pii/S1550413114005658, which is incorporated herein by reference in its entirety; Table S3 contains the results of pyrosequencing assays to replicate the CHARM results in separate samples). The 625 genome-wide significant adipocyte DMRs have FDR q values ranging from 0.004 to 0.05. In order to determine whether the results

would replicate throughout this range, the inventors examined a subset of DMRs with levels of statistical significance that spanned from the most significant to just below the 0.05 cutoff. Mice used in the replication set were also reared on a high-fat diet but were separate from those used for CHARM. Nine mouse adipocyte DMRs were assayed by bisulfite pyrosequencing. Eight of these regions had at least one CpG showing significant differential methylation in the same direction as detected by CHARM.

[0112] Figures 2A-2B are graphical representations of data illustrating replication of mouse methylation changes in additional mice and associated gene expression changes. In Figure 2A, methylation changes observed after CHARM analysis at two genome-wide significant DMRs are replicated using bisulfite pyrosequencing. Red boxes indicate CpGs assayed in pyrosequencing. For the lower pyrosequencing plots, the y axis represents methylation, and individual CpGs are plotted along the x axis. Purple dots represent control DNA artificially methylated to have 0%, 25%, 50%, 75%, and 100% methylation.

[0113] In Figure 2B, gene expression changes for genes near genome-wide significant mouse adipocyte DMRs. RNA levels were normalized to same-sample 18S RNA measurements and are displayed as $(C_T [\text{high-fat samples}] - C_T [\text{low-fat samples}])^2$. Error bars represent standard error of the C_T differences between groups. * $p < 0.05$, ** $p < 0.005$. The direction of the genome-wide significant CHARM DMR closest to the gene is denoted below the gene names; + and - represent regions hyper- or hypomethylated in the high-fat samples, respectively. See also Figure 8 for whole-genome gene expression correlations and Table 6 and Table 7 for pyrosequencing and tissue purification, respectively.

[0114] Although these were fractionated cells under investigation, to further ensure that the results were not due to cell-type shifts in the high-fat-fed obese mice resulting from the infiltration of immune cells into adipose tissue, the inventors used quantitative PCR (qPCR) to characterize the expression of multiple macrophage- and adipocyte-specific markers in the purified adipocyte samples from low-fat-fed and high-fat-fed mice. The inventors saw no significant change in the levels of expression of the macrophage (inflammatory) markers *F4/80*, *Cd14*, or *Cd68*, and the inventors did see the expected obesity-related within-adipocyte changes of the adipocyte markers *AdipoQ* and *Ccl2* (Table 6).

[0115] To examine whether these methylation changes between high-fat- and low-fat-fed mice involved changes in the expression of nearby genes, the inventors used quantitative PCR to examine the expression of 13 genes near genome-wide significant DMRs (Figure 2B). The inventors used qPCR to examine mRNA from the same adipocytes and

mice that were analyzed by CHARM. Of the 13 genes examined, 9 showed significant changes in mRNA expression in the opposite direction as methylation changes (Figure 2B).

[0116] Furthermore, the inventors assessed whether these DNA methylation changes correlated with previously published genome-wide gene expression data in a similar cohort. The inventors saw significant inverse correlations between diet-related methylation changes and diet-related gene expression changes (Figures 8A and 8B). These results compare favorably to other functional analyses of discovered DMRs. Taken together, these data show that the inventors find robustly significant DMRs in mice that correlate with metabolic traits, that these DMRs replicate in separate animals, and that methylation at many of these regions appears to have a functional effect on gene expression.

[0117] Figures 8A-8B are graphical representations of data illustrating correlation of methylation and gene expression in mouse and human adipose tissue, related to Figure 2. Figures 8A-8B show the relationship between methylation and gene expression in both mouse and human adipose tissues. Gene expression data was downloaded from GEO (see Materials and Methods) and plotted against mouse adipocyte and human adipose tissue CHARM data. Y-axes are the logarithm of the fold change (logFC) of the gene expression in high-fat-fed mice and obese humans versus low-fat-fed mice and lean humans. X-axes are the DNA methylation values calculated by CHARM (see Table S1 of Feinberg et al. (Cell Metabolism 21(1):138-149 (2015))) for the high-fat versus low-fat mouse and obese versus lean human comparisons. Here, higher values indicate hypomethylation in high-fat / obese samples. P-values are for Pearson product-moment correlations versus a null hypothesis of no correlation.

[0118] Mouse DMRs Replicated Evolutionarily in Human Adipose Tissue

[0119] The inventors reasoned that many functionally relevant DMRs in mice exposed to a high-fat diet serve an important metabolic function that would be conserved across species and often susceptible to similar environmental cues. Therefore, to determine whether the methylation changes observed in mouse adipocytes could be replicated in an evolutionarily divergent cohort, the inventors performed CHARM analysis on human subcutaneous adipose tissues from 7 lean subjects and 14 obese, sex-matched, insulin-resistant subjects of the same age range, as well as 8 obese subjects post-RYGB.

[0120] The inventors first examined the replication of mouse adipocyte DMRs in human adipose tissue from obese versus lean. The inventors observed very strong overlap between DMRs in human obese versus lean tissue and DMRs in high-fat-fed versus low-fat-fed mouse

adipocytes (all $p < 10^{-15}$, Figure 9A, rightmost five bars), showing that there is a strong correlation between areas that are regulated by methylation in metabolic dysfunction in both mice and humans.

[0121] Figures 9A-9C are graphical representations of data illustrating significance of methylation change overlap between mouse and human tissues, related to Figure 3.

[0122] In Figure 9A, all 25 mouse analyses (x-axis) are compared against the human adipose obesity analysis. Values plotted represent the largest $-\log(p\text{-value})$ for chi-squared tests for the overlap for all DMRs with nominal $p\text{-values} < 0.05$ between the given mouse analysis and the human adipose obesity analysis. In Figure 9B, for each square, the proportion of conserved mouse and human regions that had directionally consistent methylation changes in adipose tissue between species was calculated. Regions were required to have mouse and human methylation changes at or below the indicated Q-value for mouse and P-value for human. The color indicates the proportion of directionally consistent regions, with darker colors indicating a higher proportion. In Figure 9C, the observed versus expected T-statistics for the proportion of overlap between the CHARM pancreatic islet mouse methylation data and the previously reported Illumina Infinium 450k BeadChipTM pancreatic islet human methylation data.

[0123] Next, in order to determine which mouse methylation changes would replicate in human, the inventors determined that out of a total of 625 genome-wide significant mouse adipocyte DMRs, 576 had homologous regions on the human genome (hg19), calculated via the liftOver UCSC tool, and 497 had human CHARM probes within 5 kb. This is a remarkably high fraction (86.3%), suggesting that the assay method, CHARM, is highly comprehensive, and also that the location of CpG regions is strongly conserved in evolution. Of the 497 conserved DMRs, 249 (50.3%) showed significant differential methylation ($p < 0.05$) between obese and lean people (Table 7). These numbers were similar when analyzing differential methylation before and after RYGB surgery (227 out of 497). As a final restrictive step in using human methylation to validate the mouse results, the inventors determined that 170 (68%) of these regions had a consistent direction of methylation change between high-fat-fed obese mice and obese humans, such that if a particular region had higher methylation in high-fat-fed mice, that region would also have higher methylation in obese humans and vice versa.

[0124] When more restrictive human methylation significance cutoffs are used, the percentage of regions with consistent directionality (true positive rate) rises, but the total

number of retained regions drops, with 67/77 (87%) directionally consistent at human obesity p values < 0.005 , and 25/25 (100%) consistent at p values < 0.0005 (Figure 9B). All 170 directionally conserved regions were associated with the metabolic phenotypes of fasting glucose, GTT, and/or ITT in addition to mouse diet status. Furthermore, 134 of these regions had consistent directions of methylation change between both lean-obese and pre-/post-RYGB samples (e.g., higher in obesity and presurgery and vice versa), and a further 105 had postsurgery methylation values that were in between lean and presurgery methylation values, i.e., regions where methylation in obese subjects appeared to revert toward a lean phenotype after surgery (enrichment $p = 2.8 \times 10^{-3}$).

[0125] In Figure 3, the inventors present two regions that have significant methylation changes in human adipose tissue, are in homologous regions of the genome as mouse DMRs, are directionally consistent with the mouse DMRs, and have human postsurgery methylation levels that have moved closer to the lean phenotype. These regions are over two genes: *ADRBK1* (adrenergic, beta, receptor kinase 1, Figure 3A) and *KCNA3* (potassium voltage-gated channel, shaker-related subfamily, member 3, Figure 3B).

[0126] Figures 3A-3B are graphical representations of data illustrating overlapping methylation changes in human and mouse adipose tissue. For Figures 3A and 3B two genome-wide significant DMRs found in mouse adipocytes (top panels) over *Adrbk1* (A) and *Kcna3* (B) are shown along with the corresponding methylation changes in human adipose tissue (bottom panels). For the panels denoting methylation, each point represents the methylation level from an individual mouse or human at a specific genomic location, with smoothed lines representing group methylation averages. y axis, methylation values. Below each methylation plot is a panel showing genomic coordinates for the respective species and any genes at those coordinates. See also Figure 9 for tissue and species overlaps and Table 8 and Table 9 for conserved adipose mouse DMRs in human and for enrichment between DIAGRAM and conserved DMRs, respectively.

[0127] The inventors also assessed whether the human adipose DNA methylation changes correlated with previously published human genome-wide gene expression data from obese and lean individuals. As with the mouse data, the inventors saw a highly significant inverse correlation between obesity-related methylation changes and obesity-related gene expression changes (Figures 8A and 8B, right panels).

[0128] The inventors performed a similar mouse-human comparison in pancreatic islets using published DNAm data from T2D and control subjects, showing that 67% (odds ratio =

7.2, $p = 7.2 \times 10^{-6}$) of the mouse pancreatic islet DMRs that replicated in the human data had methylation change in the same direction and that these probes were far more associated with human T2D status than the rest of the probes on the array ($p = 1.18 \times 10^{-9}$, Figure 9C), demonstrating that the mouse-derived islet DMRs are enriched for potential epigenetic alteration in human T2D. Finally, the inventors also validated multiple mouse hepatocyte DMRs in human liver tissue, with 62.5% replicating (see Table S3 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015))).

[0129] Genetic Risk Loci Association with Overlapping Regions of Human and Mouse Methylation Changes

[0130] The inventors incorporated data from human GWAS for T2D using two complementary approaches that allow further characterization of the candidate obesity-related DMRs. GWAS summary statistics were obtained from the DIAGRAM (Diabetes Genetics Replication and Meta-Analysis) T2D genome-wide association meta-analysis, comprising data from 12 separate GWAS studies totaling 12,171 T2D cases and 56,682 controls (available on the World Wide Web at diagram-consortium.org). The inventors first directly explored the association between genes with obesity-related DMRs and genes conferring clinical genetic risk for T2D by calculating statistical enrichment of the GWAS regions overlapping the DMRs. The inventors found marginally significant enrichment for adipose DMRs among at least marginally significant GWAS signals (GWAS p value cutoffs starting with $p < 10^{-6}$, corresponding to enrichment p values ranging from 0.0048 to 0.0165, Table 8). Given the small number of directly overlapping regions, these results are likely strongly influenced by the strength of the *TCF7L2* signal. While much of the early literature on *TCF7L2* focused on its role in pancreatic islets, there is growing evidence that extrapancreatic effects may contribute to the T2D phenotype at this locus.

[0131] The inventors further examined statistical enrichment in the context of regulatory networks involving genes implicated in GWAS. Genes at 23 genome-wide significant GWAS signals (usually the gene nearest to the lead SNP) were directly (one-step) connected to genes near DMRs either by transcriptional control or direct protein-protein interaction (Figure 4A). This amount of interaction represents significantly more than expected by random chance ($p = 0.0206$) (Figure 10) and demonstrates how genes implicated by methylation appear to be acting in the same pathways as genes implicated by GWAS. Similarly, expanding beyond one-step connections, many of the 30 regions implicated by both methylation data and

GWAS are connected to genes identified by the mouse-only and human-mouse analyses and act in the same pathways (Figure 4B).

[0132] Figures 4A-4B are diagrammatic representations of the interactions between epigenetically conserved and genetically associated genes implicated in this study. The data represented in the Figures was generated using QIAGEN's Ingenuity IPATM (Ingenuity Systems), and these diagrams represent the connections between genes implicated in the analyses. In Figure 4A, genes with genome-wide significant linkage to T2D in the DIAGRAM meta-analysis were connected to genes near directionally conserved cross-species DMRs. Genes with no connections were dropped. In Figure 4B, starting with a set of 23 genes near T2D-associated directionally conserved cross-species DMRs, this network was grown by adding genes near species-conserved and mouse-only genome-wide significant DMRs in order to represent one potential regulatory network. Gene colors explained in within-figure legend. See also Figure 10 for the permutation analysis of the enrichment of interactions in Figure 4A.

[0133] Figure 10 is a graphical representation of data illustrating enrichment of connections between genes implicated by methylation and genome-wide significant GWAS genes, related to Figure 4. This figure shows expected and observed connections and (both direct protein interactions and transcriptional control) and overlap between genes near species conserved adipose and islet DMRs and genes with genome-wide significant linkage to T2D in the DIAGRAM GWAS meta-analysis. The set of all possible one-step connections to the DIAGRAM GWAS genes was pulled from the Ingenuity Knowledge BaseTM, and the GWAS genes themselves were added. 100,000 permutations of random genes near DMRs were overlapped with this set, and the number of overlaps from the permutations are represented by the histograms. The actual number of observed DMRGWAS connections is denoted by the vertical red line, and the p-values represent permutation p-values for the difference between observed and expected connections.

[0134] Given these results, the inventors sought to further filter the obesity-related DMRs down to the subset of genes likely associated with T2D. The inventors hypothesize that DMRs that overlap associated marker SNPs for T2D can identify genes with epigenetic mechanisms of risk in adipose tissue. As many of the DMRs overlapping GWAS T2D loci with low p values implicate genes already known to be involved in T2D, obesity, and related phenotypes, the inventors therefore selected the subset of DMRs within genetic loci that had at least marginal statistical association with T2D clinical risk.

[0135] This approach reduced the 170 regions of directionally consistent and evolutionarily conserved methylation change in adipose tissue using the SNP-level summary statistics of the DIAGRAM analysis. In all, 30 cross-species and directionally conserved adipose DMRs directly overlapped with 27 marker SNPs (or close proxies with linkage disequilibrium > 0.8) that had some evidence of association with T2D (at least $p < 0.01$, Table 2; see Experimental Procedures). The inventors also identified ten regions where conserved pancreatic islet DMRs overlap with DIAGRAM SNPs (Table 9).

[0136] In these final 30 regions, not only have the inventors connected methylation change to obesity-induced metabolic phenotypes across two species, but the association with T2D-associated SNPs also provides a candidate mechanism for the methylation changes observed in human obesity and RYGB surgery. These 27 identified SNPs could potentially explain up to 2.69% of genetic T2D liability, though only one of these loci reached genome-wide significance in DIAGRAM. Even excluding this GWAS-positive loci (*TCF7L2*), which explains 1.12% of the variance alone, the remaining regions could explain up to 1.57% of genetic variance in T2D susceptibility. These data suggest that for at least some of these loci, genetic variation underlies changes in methylation that are causal for T2D risk. It is also possible that these regions are also susceptible to environmental factors that influence local methylation and that they therefore serve to integrate genetic and epigenetic effects.

[0137] Note that this filtering-based approach is independent of assessing the statistical enrichment of T2D GWAS signal, either at SNP or gene level, within the cross-species obesity-associated DMRs, an approach commonly used with GWAS summary statistic data. This approach therefore does not diminish the potential function of genes with GWAS-positive statistical association for T2D or of the DMRs that do not overlap with GWAS-associated SNPs, for contributing epigenetically to obesity.

[0138] The inventors hypothesized that one mechanism by which DNA methylation and genetic variation contribute to T2D risk may involve enhancer activity. Using publicly available human enhancer maps in 86 independent cell and tissue types, the inventors found that a striking proportion of DMRs mapped to adipose nuclei enhancers and superenhancers (which had the largest degree of overlap across all cell types). While the background proportion of overlap for CHARM was 17.2% for adipose enhancers and 3.8% for super enhancers, 40.6% (69 overlaps, $p = 1.58 \times 10^{-15}$) and 14.7% (25 overlaps, $p = 5.72 \times 10^{-13}$) of the directionally consistent 170 regions and 53.3% (16 overlaps, $p = 5.65 \times 10^{-7}$) and 20% (6 overlaps, $p = 3.24 \times 10^{-5}$) of the further 30 GWAS-associated regions above lie in adipose

enhancers and super enhancers, respectively (Table 10). Thus, a major mechanism for methylation-mediated metabolic dysfunction is likely through epigenetic modification of enhancers. Note that most of these enhancers were not previously known to be related to T2D through conventional GWAS or other methods.

[0139] Functional Analysis of Genes Implicated by Cross-Species Methylation

[0140] In order to establish that the cross-species method can identify functional genes implicated in obesity, insulin resistance, T2D, and related research, the inventors functionally assayed five genes. The inventors selected genes with no prior association with metabolic phenotypes and that had methylation reversion after RYGB. As RYGB is a targeted, environmental therapy that improves multiple deleterious phenotypes including insulin sensitivity, the inventors hypothesized that this subset of the results would be the most likely to have an effect on T2D- and obesity-related phenotypes. The inventors then examined the physiological effect of altering the expression of these genes on adipocyte cell culture models using insulin-stimulated glucose uptake assays. This procedure can measure the responsiveness of adipocytes to insulin, a phenotype disrupted in obesity. The inventors assayed seven 3T3-L1 adipocyte cell lines, each stably expressing shRNAs or expression plasmids corresponding to one of the five selected genes or a suitable control. In order to mimic the effects of a high-fat diet, genes hypermethylated in high-fat adipocytes were knocked down, and genes hypomethylated were overexpressed. Significant changes in glucose uptake were found for four of these five (Figure 5B). Potential roles for all of these genes in modulating insulin sensitivity and resistance are considered in the Discussion below.

[0141] Figure 5A-5C are graphical representation of data illustrating overexpression and shRNA-mediated knockdown of selected genes in 3T3-L1 adipocytes. For Figures 5A and 5B, selected genes from the set of 30 species conserved and T2D-SNP overlapping adipose DMRs were either stably overexpressed (A) or knocked down with shRNA (B). Glucose uptake is plotted as fold difference from normal, error bars represent standard error, and significance was determined by two-way ANOVA modified by Bonferroni correction denoted as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Figure 5C shows DNA methylation and gene expression levels for high-fat-fed mice and obese human versus low-fat-fed mice and lean humans (e.g., “↓” indicates hypomethylation/lower gene expression in high-fat-fed and obese compared to low-fat-fed and lean). Bold arrows indicate significant changes.

[0142] Discussion

[0143] In mouse, the inventors identified 625 genome-wide significant DMRs that correlate with diet-induced obesity phenotypes in adipocytes. Of these regions, 249 had significant conserved methylation changes in human obesity, and 170 of these had the same direction of methylation change in both species. Thirty of these DMRs also overlapped with SNPs or nearby proxies that have been associated with human T2D genetic risk. These data show that DNA methylation changes in metabolic disease are conserved across species and that this conservation overlaps genomic regions where genetic polymorphisms have been associated with T2D. The approach combines three lines of evidence (epigenetic dysregulation following high-fat diet in mouse, epigenetic directional consistency in humans, and some evidence for clinical risk of T2D) to identify genes likely functionally implicated in the pathogenesis of T2D specifically through epigenetic mechanisms related to obesity.

[0144] In the present study, while the inventors use nominal p value significance to identify human methylation and GWAS results, the inventors first perform a multiple comparison correction in the initial set of mouse DMRs using a false discovery rate algorithm. As there is a growing awareness that the cumulative effect of common SNPs with low minor-allele frequency scores potentially explain large amounts of phenotypic variability beyond that of genome-wide significant SNPs identifiable by GWAS, approaches like ours that can use alternative methods to identify significant areas of potential genetic risk are necessary. The unique SNPs in these regions potentially account for 2.76% of T2D genetic variance, almost half of which is known by purely genetic analysis and may be epigenetically mediated.

[0145] The inventors observed significant changes associated with 4 out of 5 genes assayed by insulin-stimulated glucose uptake assay, a common indicator of insulin resistance. Screens using this assay and performed on sample sets not enriched for genes in glucose-insulinemic pathways have found a far smaller percentage of genes that will alter glucose uptake (~ 10%), indicating that the method can successfully select potential targets with a much higher than random probability of affecting insulin sensitivity.

[0146] Three of the genes that the inventors found had altered glucose uptake fell into the classical inverse methylation-gene expression correlation: *Mkl1*, *Plekho1*, and *Tnfrsf8l2* were all hypomethylated in high-fat-fed mice and obese humans, had increased gene expression in corresponding subjects, and, when these genes were overexpressed in cell culture adipocytes, exhibited decreased glucose uptake in response to insulin, which would fit with the increased

insulin resistance commonly observed in obesity and diabetes. While none of these genes has previously published roles in insulin resistance, several have suggestive links to metabolic phenotypes. *Mkl1* is known to be a transcriptional coactivator of serum response factor (SRF), which been associated with insulin resistance in skeletal muscle. Similarly, PLEKHO1 has recently been shown to inhibit AKT/PI3K signaling, a pathway known to be involved in insulin signaling. With regards to the direction of glucose uptake change, the inventors note that insulin signaling induces both positive and negative feedback within affected cells, and without a methylation-gene expression candidate mechanism it is not possible to determine which feedback loop the methylation changes are involved with.

[0147] It is worth noting that as these genes did not contain common variants that passed the genome-wide significant GWAS threshold, they would not have been identified by GWAS alone. Similarly, only 4 out of these 5 genes had significant gene expression changes. This functional assay illustrates how the method of combining cross-species methylation data with GWAS results for common SNPs can implicate genes that would not have been detected otherwise.

[0148] Recent work in the laboratory has identified regions of the genome where DNA methylation acts to mediate a genetic effect on rheumatoid arthritis, and the methylation changes in obese humans could potentially act in an analogous role. The results in obese and insulin-resistant mouse models, however, identify methylation differences even between inbred mice and thus are definitively the result of environmental stimuli rather than a genetic underpinning. The fact that the inventors see many of these same methylation changes in obese humans, and that these changes are located over regions with known genetic links to T2D, implies that DNA methylation levels could be integrating and mediating genetic and environmental causes of metabolic disease at specific genomic loci.

[0149] It is encouraging that many of the genes described here show pathway relationships to known genetic associations (Figure 4). For example, *PRCI*, a regulator of cytokinesis, is associated with T2D by a genome-wide significant DIAGRAM result, but it has no known connection to any other gene implicated by genome-wide significant DIAGRAM loci. Its transcription, however, is regulated by FOXO1, an important transcription factor in gluconeogenesis, insulin signaling, and adipocyte differentiation that the inventors find to be differentially methylated in both mouse and human obesity. FOXO1 is in turn regulated by TCF7L2, one of the strongest GWAS results. Furthermore, combining genes from all levels of this study creates potential regulatory networks that include genes

with known involvement in T2D, but also incorporate closely connected genes with no previously known obesity or T2D association that are shown to be involved with obesity and insulin resistance in this story (Figure 4B). Some of these genes, such as *FASN* and *APP*, appear to be loci in this network and could represent potentially important targets.

[0150] There are many approaches for and important applications of interrogating the association of functional and genetic elements using GWAS summary statistics (ENCODE Project Consortium, 2012), but the approach is unique in its leverage of carefully controlled biological systems to directly integrate cross-species functional epigenomics and clinical genetic risk by stratification. This work, of course, does not address or diminish the many GWAS associations that are not associated with methylation changes. Additionally, it is important to note that while the inventors do not directly address the issue of methylation causality in this study, causality is, at the least, multi-tiered. The functional data certainly indicate that these epigenetic changes are functionally proximate to T2D-relevant phenotypes and therefore important for discovery and for clinical translation. Current systems biology literature challenges conventional notions of causality as there is both positive and negative feedback in most complex living systems.

[0151] The approach described in this study may have broad applicability to identify candidate genes that may better dissect mechanisms and potential routes of treatment in common human disorders, such as cancer and cardiovascular disease. The accessibility of a limited cohort of relevant patients with well-characterized clinical materials before and after disease exposure is plausible for cross-species replication. This type of analysis can generate a reliable, functional candidate disease gene set that can be used to interrogate SNP data sets and lend additional support to specific targets that would not ordinarily pass the genome-wide correction threshold. The end result is a process that can integrate information from multiple complementary sources to identify potential targets essential for the pathogenesis of common diseases, such as obesity or T2D, that do not involve highly penetrant single genes, but rather arise from multiple defects along pathways that integrate genetic, epigenetic, and environmental cues.

[0152] Tables**[0153]** Table 1. Genome-wide significant mouse DMRs.

Tissue	Analysis	q val < 0.05	q val < 0.1
Adipocytes	Diet	232	448
	Weight	183	288
	Fasting glucose	235	571
	GTT	0	3
	ITT	294	419

[0154] q values generated based upon comparison of observed DMR areas to areas generated by 1,000 random permutations of phenotype/methylation associations. See also Table S1 of Feinberg et al. (*Cell Metabolism* 21(1):138-149 (2015)) for a full list of all mouse DMRs.

[0155] Table 2. Mouse-human DMRs with genetic T2D risk loci association.

Gene name	Relative location of DMR	Distance to TSS	RYGB reversion	DIAGRAM p value
Tcf7l2	inside intron	43,058	–	4.90E-68
Tcf7l2	Inside intron	77,345	–	4.90E-68
As3mt	overlaps 5'	0	+	9.60E-06
Etaa1	Inside intron	618	+	4.70E-05
Tnfsf8	overlaps 5'	0	–	0.00029
Plekho1	overlaps exon	4,965	+	0.00045
Tnfaip8l2	inside intron	337	+	0.00045
Akt2	inside intron	20,427	–	0.00049
DIAGRAM GWAS 0.001 cutoff				
Lhfp12	Inside intron	2,490	+	0.001
Mkl1	overlaps 5'	0	+	0.0014

Gene name	Relative location of DMR	Distance to TSS	RYGB reversion	DIAGRAM p value
BC048644 (Car5a)	overlaps exon	146	+	0.0015
Rgs3	downstream	10,8842	+	0.0019
Fgd3	Inside intron	11,100	+	0.002
Stau1	overlaps 5'	0	+	0.0022
Tmcc3	Inside intron	43,772	+	0.0025
Tbx3	inside exon	12,714	-	0.0029
Gstz1	Inside intron	10,332	+	0.0029
Taok3	Inside intron	549	+	0.0036
Bnip3	Inside intron	1,863	—	0.0039
Dlst	overlaps 5'	0	+	0.0053
Kcna3	Close to 3'	2,192	+	0.0064
Cln8	Inside intron	3,055	+	0.0065
Cd37	exon	2,687	+	0.0069
Nfib	Inside intron	100,380	—	0.0071
Pck1	promoter	453	+	0.0072
Pck1	overlaps 5'	0	+	0.0072
Pcx	inside intron	59,049	+	0.0073
Hoxd3	inside intron	7,307	+	0.0084
Cd33	overlaps 5'	0	+	0.0087
Evl	exon	157	+	0.0099

[0156] Shown are the names of the nearest gene to the mouse and human differential methylation, the position of the DMR relative to the gene, the distance to the transcriptional start site (TSS), whether the direction of methylation change (sign of smoothed effect statistic) post-RYGB surgery reverts toward lean subject methylation levels (RYGB

reversion), and the p value of the T2D genetic association in the region. See also Table 9 for an analogous table with the pancreatic islet results instead and Table 10 for conserved adipose DMRs that overlap with adipose enhancers.

[0157] Table 3. Pyrosequencing primers.

Pyrosequencing Primers	Sequence	SEQ. ID. NO.
Runx1 Long 5'	TTGAGTTTGTTAAATTTAGGGGTAAGT	1
Runx1 Long 3'	TTCAAACAACATTTTAAATCATTTTC	2
Runx1 Nested 5'	TTGAGTTTGTTAAATTTAGGGGTAAGT	3
Runx1 Nested 3'	/5Biosg/CAAACAAAACTATTA ACTCTAAAACCAC	4
Runx1 Sequencing 1	GTTATTAGAGTAAGTGTA	5
Runx1 Sequencing 2	GATAAAATTTAAAGAGTGTT	6
Runx1 Sequencing 3	GTTTAAGTATTTATTA AAAATAT	7
Pscdbp Long 5'	TTTTTTAGGGAAAAGAATTTTTTTT	8
Pscdbp Long 3'	ATCTCAAACCACAACAATCACATAA	9
Pscdbp Nested 5'	GGTATTATTTTTAGTAGAGGTTAGGTAAA	10
Pscdbp Nested 3'	/5Biosg/CAAAACCAAAAAACCATATATTAAC	11
Pscdbp Sequencing 1	GGTATTATTTTTAGTAGAGGTTAGGTA	12
Pscdbp Sequencing 2	ATAGTGTTTGTTAGTATTTGAAT	13
Stap1 Long 5'	TTTTATAATAATTGAAGGAGGGAAAGT	14
Stap1 Long 3'	AAAAACAATATAACCCAAACAAAAAC	15
Stap1 Nested 5'	TTTTATAATAATTGAAGGAGGGAAAGT	16
Stap1 Nested 3'	/5Biosg/TACACACTTACCTAATAATCAAACC	17
Stap1 Sequencing 1	GAATAGTTGTTTTTTTTTTTAATAT	18
Stap1 Sequencing 2	AATTTTAAAGTAAAGGTTATG	19
Pck1 Long 5'	TGGTAAAGGTTTTGTTGTTTAAGTGT	20

Pck1 Long 3'	ATTCTCTAACCATCCCAAAATAAAC	21
Pck1 Nested 5'	TTTTTAGATATTTGGGTATTTAAGA	22
Pck1 Nested 3'	/5Biosg/ACTATAAACTTTATTCTAACAAAACAATAC	23
Pck1 Sequencing 2	GTTTGATGTATATTTTTTTTG	24
Pck1 Sequencing 3	TTTAGAGTAGGGGTTAGTAT	25
Dguok Long 5'	AGTTGTATAATTTATTGTGGGTGG	26
Dguok Long 3'	TCCTTTAAAACTTCCTCAAACATTT	27
Dguok Nested 5'	AGTTATGGAGGGTTAATTTGTGTTT	28
Dguok Nested 3'	/5Biosg/TCCTTTAAAACTTCCTCAAACATTT	29
Dguok Sequencing 1	GTTAATTTGTGTTTGGGATT	30
Dguok Sequencing 2	ATTTTLAGAGGATTGGGTGAAG	31
Dguok Sequencing 3	GGAGTTATTGATAAATTT	32
Kcnj11 Long 5'	AGAGTTTAGGTTATAGGTGGGAGGT	33
Kcnj11 Long 3'	AAAATATCCTACCAACCAAAAAAAAA	34
Kcnj11 Nested 5'	TTTAGGTTATGTTTAAGGGTTTTGG	35
Kcnj11 Nested 3'	/5Biosg/AAAACTAAAAAACCCACACAAACAC	36
Kcnj11 Sequencing 1	ATTTTTTTTTTTAGTAATTTAGATAAG	37
Kcnj11 Sequencing 2	ATTTTATTTTTTTAGTTTTTTGG	38
Kcnj11 Sequencing 3	GAGGTGAGTTTAGGTAGATTT	39
Zfhnx2 Long 5'	TTTTGAGGGTTTAGTGGTAGTTTGT	40
Zfhnx2 Long 3'	CAAATTCAATAAAAAACACAAATTAATAAAA	41
Zfhnx2 Nested 5'	AGGGTTTAGTGGTAGTTTGTTAGGTTAT	42
Zfhnx2 Nested 3'	/5Biosg/AAAAAAAATCAACAAAAACAATATTAAATT	43
Zfhnx2 Sequencing 1	AAATTGTTTATTTTTTTGTAG	44
Zfhnx2 Sequencing 2	TTTTTTATTTTATTTTATTTTTT	45

Slc38a4 Long 5'	GGAGTTTTTATTAGGAGAGGGTGTAG	46
Slc38a4 Long 3'	ATAAACCCAAAATAACCTCCAACCTCA	47
Slc38a4 Nested 5'	TATTTTGATGGTGTAGAGATGAATTT	48
Slc38a4 Nested 3'	/5Biosg/CCACCACCACCTAAAAAAAACCT	49
Slc38a4 Sequencing 1	ATGGTGTAGAGATGAATT	50
Slc38a4 Sequencing 2	GTATAGTTATATTTTAAAT	51
Slc38a4 Sequencing 3	TTATTTGATGTTTAATATATTT	52
Chpt1 Outer F	GGGAGTTTAGAATAGTGATTGGTTG	53
Chpt1 Outer R	TCCCTTCCTTAAATAACCTTCCTAC	54
Chpt1 Inner F	GGGAAGATTTTTAAATAGAGAGGATGT	55
Chpt1 Outer R	/5BiosG/TACCCTAAAACTAAACCCCAAAC	56
Chpt1 Seq2	GATATTTAATATAATATTTATTT	57
Chpt1 Seq3	ATTGAGTTTTAGGTTAGTTTAA	58
Chpt1 Seq4	TTGGGGGAATTAATTTAAGT	59
Liph Outer F	ATAGTTTAGGAGGAAAGTTGGTGTT	60
Liph Outer R	AAAAACTTCCAAAACATAAAAAAAA	61
Liph Inner F	AAGGGATTTAAGGGATTTTTAAATTT	62
Liph Inner R	/5BiosG/CAACTCCCTACAACAAAACACTTT	63
Liph Seq1	GGTAGTTTGGTTTATTT	64
Liph Seq2	GATTATTTAGATGTTTGT	65
Liph Seq3	GTGGTATGATAGGTAGTAG	66
Fasn Outer F	GGGTTTTAAGAGGTTGTTGGTTAAT	67
Fasn Outer R	AACCCTAAACAAAACACAATTCAC	68
Fasn Inner F	TAGTTTGTTTTGGGATAGGTTGTG	69
Fasn Inner R	/5BiosG/AATATACCCCCAAAAATAAAAAACC	70

Fasn Seq1	GAATATAAAGGTAAAGTGTTA	71
Fasn Seq2	AGAGTTTGGGTAGTTAGATAG	72
Fasn Seq3	AGGGTTTTTATTTTATTAAG	73
Fasn Seq4	GTTAATTAAATTTTTTAATTG	74
Axin2 Outer F	GATTTTAAAGGAGGGGATTTGTAG	75
Axin2 Outer R	CTATCCCACATCACCAATCTAACT	76
Axin2 Inner F	TTTTTTTATTTTATGTGGTGTTGT	77
Axin2 Inner R	/5BiosG/ACTAAACTATCCCTACCTATTCCTC	78
Axin2 Seq1	TTTTATTTTATGTGGTGTT	79
Axin2 Seq2	TAATTGTTTTTGTGTTTTG	80
Axin2 Seq3	GAATTTTAGAGTGAGGATTG	81
Scd1 Outer F	AGAAGGTTTGGGGTAATATAGAAGTTT	82
Scd1 Outer R	AAATCCCCTCTCCTTAAACATAAC	83
Scd1 Inner F	TGGGAAATTTTTTGATAGTT	84
Scd1 Inner R	/5Biosg/AATTCTACTAAATCCTCAAAAAAACTAAAC	85
Scd1 Seq1	TTTGGGTTATATATGTGTTA	86
Scd1 Seq2	GTTTTTGTATTTGTGAGGG	87
Scd1 Outer F	AAGGGAGGTTTTTGTTATTTATTTA	88
Scd1 Outer R	ACCTTCCTTATAACCATCAATTCC	89
Scd1 Inner F	TGTTTTTTAGTAAGTGAGAAGAGATGGT	90
Scd1 Inner R	/5Biosg/AACCAAATTTAAACCCAACCTAAAC	91
Scd1 Seq1	GTGGTTTAGAAAGAAGAGTTTTGT	92
Fermt2 Outer F	TGTGGTAAGGTTTATTTTTTAGAGG	93
Fermt2 Outer R	AAACAACTTTTATCTCCCCTTTAC	94
Fermt2 Inner F	ATAATAGAGGATAGAAATAAAAGAATGAAA	95

Fermt2 Inner R	/5Biosg/CCTTCAAATTATATAAATTTCAAATAATAA	96
Fermt2 Seq1	ATTTTAGAAGTTATAATTTAAGTA	97
Atp6v0a1 Outer F	TTTTGGAATTAGTTTAAAAGGGTTG	98
Atp6v0a1 Outer R	AACAAAAAACAAAACAAAACCAAAT	99
Atp6v0a1 Inner F	ATTTTAAAGTAGGGATTTTTTTGTGAG	100
Atp6v0a1 Inner R	/5Biosg/TCATCCTAATACCTTCAAACACTCTC	101
Atp6v0a1 Seq1	GATAAGATTTTAATGTATTTAAGTT	102
Arhgap29 Outer F	TATGGATTTTGGGATTTTGTATTAT	103
Arhgap29 Outer R	TACAACAACCTAACCAACAAAAAAA	104
Arhgap29 Inner F	TTTAAATTTTGAATTTAGAGGAAATTTAGT	105
Arhgap29 Inner R	/5Biosg/AAAATATTTAATAAATTTCTATTCCCCC	106
Arhgap29 Seq1	GTTATTTGTATTTTGTAAATT	107
Arhgap29 Seq2	TTGTAAAGTGTTTGTGATAA	108
Arhgap29 Seq3	ATTAGATATTTTGTATAATTT	109
Masp1 Outer F	TATGTGTATTTATATTTGGGATTTTTTTAG	110
Masp1 Outer R	AATAAACTCTTCTAACCCCTAACTC	111
Masp1 Inner F	GAAGTTTGTGTTGTTTGTATTTTG	112
Masp1 Inner R	/5Biosg/TCTATATTTACTTAAACATACCCTC	113
Masp1 Seq1	GGTTTTGGTGTTTTTGGAGTGGGAGA	114
Masp1 Seq2	GTTATGTAGTAGGTGAAATGAGTT	115
Elovl5 Outer F	TTGTTGTATAGGTTTGTAGTTTAGGAGTAA	116
Elovl5 Outer R	TCAAAAACCCAATTAAATCAATATTC	117
Elovl5 Inner F	AAAATTGTTTAAGAGTATATTTTTTAAAAA	118
Elovl5 Inner R	/5Biosg/AACATCCAACATTAATTTCTTACC	119
Elovl5 Seq1	ATTTTTATTGTAAAGTTATATATGATT	120

Elov15 Seq2	TAGAGGTTTTTTTAAAGAATGTG	121
Elov15 Seq3	ATTTTGTAATTAATTAGATGG	122
Tmem140 Outer F	TATAGAATGATGTTTATAAAGTGGGATATA	123
Tmem140 Outer R	AATTAAAAACCCCATACACTCTTCT	124
Tmem140 Inner F	GGGATATAAATATATATATTTATGTAATTT	125
Tmem140 Inner R	/5Biosg/AACCAATATTTCTTCAAAAAACAA	126
Tmem140 Seq1	AGAAGTGTGTTGTTTAGAGTGGT	127

[0158] Table 4. Quantitative PCR primers.

Quantitative PCR Primers	Sequence	SEQ. ID. NO.
Tnks1bp1 F	CCCAGGACCCTCACTCCAT	128
Tnks1bp1 R	TCCCAAACCTCCCAGTCTTGAA	129
Fbxw8 F	GCCAGGTTGCCTTTGGAGT	130
Fbxw8 R	TCCCGGATGTTGACACAGGTA	131
Sorbs1 F	CCCCGTCTGAGGTAATAGTTGT	132
Sorbs1 R	GAGCAGTCTCCAGGAGTATAGTC	133
Vps13c F	GAAGCTAAAGTAAAAGCCCACGA	134
Vps13c R	ACACATCAGAGGTGTTGACAATG	135
Tcf7l2 F	AACGAACACAGCGAATGTTTCC	136
Tcf7l2 R	CACCTTGTATGTAGCGAACGC	137
Pcx F	CTGAAGTTCCAAACAGTTCGAGG	138
Pcx R	CGCACGAAACACTCGGATG	139
Tnfsf8 F	GCACAAGTCGCAGCTACTTCT	140
Tnfsf8 R	GGAGTGGAGTCCTTTTTCTGG	141
Etaal F	GGTGGCACGGGAATGAGTC	142

Etaal R	GATTTGTACTGGCGTCTCCTTT	143
Pck1 F	CTGCATAACGGTCTGGACTTC	144
Pck1 R	CAGCAACTGCCCCGTACTCC	145
Rgs3 F	GCTTCCTGTAGGACAAGACCT	146
Rgs3 R	GGCTTTGAGGGGGCTTAGG	147
Stau1 F	GGACCCTCACTCTCGGATG	148
Stau1 R	TTCTGGCAGGGGTTCACCTCT	149
As3mt F	GGGAATGTACTGAAGACATCTGC	150
As3mt R	CCACAGCCATAATACCTCGAACT	151
Akt2 F	ACGTGGTGAATACATCAAGACC	152
Akt2 R	GCTACAGAGAAATTGTTTCAGGGG	153
Tnfaip8l2 F	TCAGCTCAAAGAGTCTGGCAC	154
Tnfaip8l2 R	GGTAAAGCTCGTCTAGCACCTC	155

[0159] Table 5. Gene ontology for genes near DMRs, related to Table 1.

DMR set	Gene Ontology Biological Process Term	Count	List Total	Enrichment Q-value
Adipocyte Hypermethylated DMRs	triglyceride metabolic process	9	47	3.33E-04
	acylglycerol metabolic process	9	56	8.23E-04
	neutral lipid metabolic process	9	58	7.51E-04
	positive regulation of cholesterol storage	4	6	2.15E-03
	low-density lipoprotein particle clearance	4	7	3.96E-03
	cellular carbohydrate metabolic process	11	131	9.12E-03
Adipocyte Hypomethylated DMRs	regulation of response to stimulus	81	2173	3.13E-05
	neutrophil chemotaxis	8	32	7.50E-04
	positive regulation of response to stimulus	48	1110	5.10E-04
	cell activation	23	334	4.70E-04
	myeloid leukocyte activation	10	59	4.13E-04
	immune system process	38	784	3.70E-04

[0160] Genes near genome-wide significant DMRs (q-value <0.05) for adipocyte-fasting glucose associations were submitted to the Gene Ontology enRIchment anaLysis and visuaLizAtion tool (GORilla) along with a background of all the genes possible to find on the applicable array. The list of genes found in adipocytes was first divided into hypomethylated and hypermethylated groups depending on the status of the corresponding DMR. Here, hypermethylation refers to areas where increased methylation is associated with higher fasting glucose and hypomethylation the converse.

[0161] Table 6. Results of qPCR assay to test adipose tissue purification, related to Figure 2.

Gene	Fold change (high fat/low fat)	SD	P-value
Adipoq	-1.41	0.4	0.039
Pparg	-0.58	0.3	0.167
Ccl2	1.29	0.32	0.043
Albumin	-0.08	1.32	0.476
CD68	1.69	0.9	0.174
Corola	0.02	1.41	0.494
F4/80	0.06	0.64	0.463
CD14	0.27	0.38	0.341

[0162] This table shows the results of the quantitative PCR assay to test if the mouse adipocyte tissue samples were pure.

[0163] Table 7. Conserved mouse-human DMRs, related to Figure 3.

[0164] This table lists the 497 mouse DMRs mappable onto the human chromosome and with 5kb of a human probe. Listed are the genomic coordinates and width for each mouse differentially methylated region (DMR), q-values for the mouse DMRs derived from false discovery rate (see methods, qval), the gene symbol nearest gene to the mouse DMR, the p-values for the corresponding changes in human obesity and surgery, and the slopes for the methylation change for both human obesity and surgery.

Chr	Start	End	Wid	qval	Nearest Gene	Nearest Gene Distance	Human Obesity p.val	Obesity Methylation Slope	Human RYGB p.val	RYGB Methylation Slope
chr1	6804060	6804159	100	0.044	Dnajc11	31055	0.05396806	0.127370809	0.076926702	0.096956819
chr1	19804495	19805076	582	0.005	Capzb	6360	0.009584786	0.102400227	0.080255224	0.065423927
chr1	23171771	23172254	484	0.006	Ephb2	123359	0.163730493	0.037281993	0.240294807	0.014835724
chr1	27681877	27682185	309	0.012	Map3k6	11634	0.017716616	0.077604526	0.009129994	0.064042072
chr1	36191885	36192039	155	0.044	Clspn	44625	0.239591101	0.036206241	0.201558347	0.045218473
chr1	37838402	37839079	678	0.032	Zc3h12a	79935	0.378536274	0.015552746	0.001183568	0.17839242

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chr1	48351513	48351750	238	0.011	0008561	93473	0.000271516	0.138474484	0.105292916	0.064390092
chr1	61909979	61910398	420	0.026	Nfia	321628	0.283317249	0.065328087	0.171060478	0.054337879
chr1	63253431	63254413	983	0.006	Atg4c	5876	0.090799505	0.029828429	0.02784476	-
chr1	64061530	64062519	990	0.006	Pgm2	1768	0.023274675	0.09603559	0.2239538	-
chr1	65733701	65734201	501	0.012	Dnajc6	39493	0.297952055	0.036724144	0.02627919	-
chr1	94153416	94153898	483	0.009	Bcar3	5588	0.641591964	0.012994303	0.358888654	0.024734722
chr1	1E+08	1E+08	515	0.005	Palmd	901	0.191059976	0.020861026	0.035225878	-
chr1	1E+08	1E+08	77	0.042	Slc35a3	565	0.045055579	0.053255223	0.373986866	0.029616686
chr1	1.01E+08	1.01E+08	465	0.005	Dbt	1015	0.026114662	0.043754768	0.266052273	-
chr1	1.11E+08	1.11E+08	489	0.009	Kcna3	2193	0.000830083	0.174664378	0.026296074	-
chr1	1.11E+08	1.11E+08	449	0.006	Kcna3	677	0.028609698	0.071486755	0.078482013	-0.10916874
chr1	1.14E+08	1.14E+08	941	0.006	Ptpn22	288	8.28E-05	0.140138463	0.111418668	-
chr1	1.14E+08	1.14E+08	609	0.005	Ptpn22	0	8.28E-05	0.140138463	0.111418668	-
chr1	1.14E+08	1.14E+08	354	0.006	Ptpn22	972	8.28E-05	0.140138463	0.111418668	-
chr1	1.5E+08	1.5E+08	244	0.022	Otud7b	424	0.481806589	0.013695731	0.038840057	-
chr1	1.5E+08	1.5E+08	305	0.012	Plekho1	4964	0.011515715	0.061527856	0.092524395	-
chr1	1.51E+08	1.51E+08	425	0.005	Tnfaip8l2	336	2.58E-05	0.203542029	0.043102817	-
chr1	1.54E+08	1.54E+08	206	0.026	S100a4	632	0.015459655	0.111432355	0.006174879	0.124908817
chr1	1.55E+08	1.55E+08	562	0.044	Flad1	888	0.063435358	0.088485589	0.062015242	-
chr1	1.61E+08	1.61E+08	692	0.005	Cd48	0	0.037990596	0.040921991	0.058606544	-
chr1	1.61E+08	1.61E+08	392	0.005	Arhgap30	636	4.80E-09	0.264158562	0.010337311	-
chr1	1.61E+08	1.61E+08	389	0.006	Fcgr3	411	0.002652877	-0.16864877	0.017223425	-

chr1	1.73E+08	1.73E+08	1177	0.005	Tnfsf4	359	0.082438786	0.056016855	0.477320041	0.015276966
chr1	1.73E+08	1.73E+08	233	0.017	Prdx6	3000	0.102115617	0.04274556	0.003004404	0.096005652
chr1	1.82E+08	1.82E+08	403	0.017	Glul	2739	0.068397296	0.062977644	0.025488547	0.098159346
chr1	1.93E+08	1.93E+08	438	0.006	Rgs1	0	0.015727132	0.133234131	0.016067624	0.102588829
chr1	1.99E+08	1.99E+08	2388	0.005	Ptprc	0	0.018214222	0.100339811	0.001093455	0.147785599
chr1	1.99E+08	1.99E+08	460	0.045	Ptprc	39084	0.407309588	0.037295152	0.278995332	-0.0199901
chr1	1.99E+08	1.99E+08	719	0.005	Ptprc	368025	0.176595525	0.042360171	0.012370438	0.11232229
chr1	2E+08	2E+08	220	0.022	Nr5a2	258072	0.163636918	-0.04654987	0.249703563	0.022766232
chr1	2.12E+08	2.12E+08	731	0.012	Nek2	658	0.01220632	0.100825287	0.061637418	0.091412309
chr1	2.12E+08	2.12E+08	1610	0.005	Ints7	45038	0.205896102	0.032334064	0.032263572	0.111604731
chr1	2.21E+08	2.21E+08	224	0.026	Mark1	51166	0.173358467	0.062705846	0.198390864	0.054456176
chr1	2.21E+08	2.21E+08	511	0.005	C130074 G19Rik	995	0.191583115	0.055482328	0.216499795	0.044018923
chr1	2.22E+08	2.22E+08	569	0.005	Dusp10	5715	0.107649879	0.033404002	0.038084354	0.038682285
chr1	2.3E+08	2.3E+08	415	0.026	Galnt2	33499	0.067540877	0.041459111	0.021183552	0.085751104
chr1	2.36E+08	2.36E+08	143	0.044	Nid1	69287	0.428590605	0.012324001	0.360883807	0.013890278
chr1	2.42E+08	2.42E+08	271	0.012	Opn3	2726	0.004056696	0.109795008	0.002465152	0.141015585
chr1	2.48E+08	2.48E+08	257	0.043	Trim58	941	0.200965059	0.040951299	0.004083849	0.148912371
chr2	10093863	10094145	283	0.036	Grhl1	6541	0.149489146	0.059018061	0.042991352	0.098039251
chr2	16084776	16085714	939	0.024	Mycn	3829	0.026462974	0.037242163	0.020034154	0.042121351
chr2	21151188	21151541	354	0.006	Apob	91845	0.006293461	0.043007646	0.079009519	0.025968826
chr2	25668139	25668364	226	0.049	Dtnb	166912	0.117496348	0.099659161	0.041252876	0.126276622
chr2	38669779	38670055	277	0.015	Arl6ip2	52758	0.192013286	0.04128462	0.164498053	0.036971722

chr2	44308569	44309760	1192	0.005	Lrppe	82575	0.183046934	0.088509005	0.36710835	0.074797085
chr2	45911195	45911602	408	0.035	Prkce	33264	0.277667197	0.043119313	0.34129699	0.035189023
chr2	46795289	46796098	810	0.009	Rhoq	23492	0.022247323	-0.09536594	0.343882477	0.042790041
chr2	47216781	47216960	180	0.024	Ttc7	34850	0.001719588	0.178933354	0.177314794	0.068088499
chr2	62426656	62427018	363	0.012	B3gnt2	2506	0.021726827	-0.05800806	0.007112103	0.124450687
chr2	63270337	63271211	875	0.006	Ehbp1	276814	0.105911246	0.032484673	0.092082131	0.025752407
chr2	67625367	67625994	628	0.011	Etaa1	587	0.014192426	0.159468397	0.057427316	0.081615983
chr2	68592241	68593052	812	0.005	Plek	0	0.000417575	0.116575262	0.001208315	-0.14693488
chr2	68961351	68961704	354	0.012	Arhgap25	233	0.000287308	0.169520485	0.009437236	0.102669374
chr2	68962521	68963097	577	0.005	Arhgap25	532	0.000287308	0.169520485	0.009437236	0.102669374
chr2	70211010	70211141	132	0.006	Asprv1	17464	0.074793806	0.040295727	0.008663913	0.040009929
chr2	80531572	80531693	122	0.044	Lrrtm1	64	0.01267564	-0.12761776	0.001646793	0.138411511
chr2	1.01E+08	1.01E+08	287	0.03	Lonrf2	156474	0.037745155	0.103856175	0.308482567	0.050985187
chr2	1.2E+08	1.2E+08	286	0.012	Dbi	4474	0.096817539	0.042198961	0.122807867	0.044198351
chr2	1.2E+08	1.2E+08	257	0.03	Sctr	1272	0.049402728	0.060074519	0.037452731	0.081224769
chr2	1.37E+08	1.37E+08	284	0.029	Cxcr4	5558	0.025691591	0.101355347	0.286970636	0.041871989
chr2	1.45E+08	1.45E+08	299	0.011	Zeb2	122956	0.013432772	0.148539776	0.433483904	0.03426897
chr2	1.58E+08	1.58E+08	1374	0.005	Pscdbp	313	0.003845787	0.126579923	0.009591686	0.113021718
chr2	1.58E+08	1.58E+08	841	0.005	Pscdbp	350	0.003845787	0.126579923	0.009591686	0.113021718
chr2	1.59E+08	1.59E+08	768	0.005	Acvr1	29817	0.345419057	0.037080746	0.380691236	0.029122073
chr2	1.77E+08	1.77E+08	325	0.033	Hoxd3	22043	0.00782395	0.119911701	0.009779601	0.072776117
chr2	2.34E+08	2.34E+08	685	0.005	Atg16l1	551	0.408179201	0.018866827	0.108141227	0.041195482
chr2	2.37E+08	2.37E+08	372	0.008	Gbx2	1887	0.014302383	0.228144487	0.001711165	0.057789286

chr2	2.42E+08	2.42E+08	227	0.006	Kif1a	30209	0.157313624	0.04528784	0.130219629	0.032731766
chr6	1608907	1609124	218	0.033	Foxc1	903	0.02149946	0.144758449	0.026549578	0.067922771
chr6	6701849	6702647	799	0.009	Ly86	113412	0.138073502	0.044960274	0.016446104	0.102655949
chr6	8069397	8069892	496	0.029	Eef1e1	17563	0.230098757	0.03717546	0.529955234	0.010570813
chr6	10557112	10557387	276	0.015	Gcnt2	657	0.084712725	0.085695391	0.051183188	0.099709759
chr6	20149392	20149620	229	0.042	Mboat1	61723	0.357755126	0.029006955	0.111157742	0.033338785
chr6	26595986	26596063	78	0.044	Abt1	849	0.001774016	0.091203913	0.029453493	0.047168264
chr6	43488870	43489065	196	0.03	Rpo1-1	3770	0.039655641	0.063665591	0.002944446	0.080512729
chr6	43977929	43978138	210	0.026	Mrpl14	112658	0.067770138	0.049733177	0.106801536	0.048325515
chr6	72001391	72001673	283	0.015	Ogfr1l	2607	0.650063432	0.021514144	0.394440329	0.045118385
chr6	75913118	75913414	297	0.011	Col12a1	2313	0.039964536	0.036258368	0.018634341	0.043387645
chr6	79922240	79922533	294	0.009	Hmgn3	27731	0.031223875	0.101608036	0.133122901	0.046937456
chr6	83774408	83774634	227	0.03	Ube2cbp	549	0.088148366	0.024298645	0.111115821	0.083926315
chr6	90995934	90996135	202	0.026	Bach2	10138	0.31033621	0.018624689	0.050662463	0.062412637
chr6	1.07E+08	1.07E+08	222	0.02	Atg5	98735	0.032929012	0.092864789	0.005575201	-0.1356305
chr6	1.08E+08	1.08E+08	261	0.011	Scml4	26	0.0002544	-0.15054077	0.0011841	0.119484281
chr6	1.1E+08	1.1E+08	632	0.006	5033413 D22Rik	26320	0.07455092	0.113810962	0.089160776	0.100483874
chr6	1.22E+08	1.22E+08	146	0.044	D630037 F22Rik	0	0.214211871	0.076381082	0.065115908	0.035362635
chr6	1.23E+08	1.23E+08	315	0.006	Hsf2	708	0.782800905	0.005988991	0.090043395	0.090159621
chr6	1.36E+08	1.36E+08	265	0.026	Ahi1	69750	0.150413734	0.027839173	0.071477084	0.028903577
chr6	1.36E+08	1.36E+08	283	0.026	Pde7b	192747	0.138900626	0.033768848	0.031368491	0.061275681
chr6	1.4E+08	1.4E+08	525	0.005	Cited2	2998	0.071358568	-0.05626553	0.0250784	0.088202576
chr6	1.43E+08	1.43E+08	221	0.028	Gpr126	69582	0.060847098	0.063670558	0.005923013	0.077463306

chr6	1.55E+08	1.55E+08	303	0.032	A130090 K04Rik	72682	0.054281324	0.064447241	0.016348248	0.072805909
chr6	1.64E+08	1.64E+08	727	0.005	Qk	17101	0.005485027	0.144576733	0.039999443	0.095803967
chr6	1.65E+08	1.65E+08	479	0.005	Qk	569295	0.06111785	0.049408781	0.072372323	0.043504723
chr8	1715132	1715419	288	0.012	Cln8	3056	0.020312359	0.055086638	0.1977797	0.031975276
chr8	17529635	17529755	121	0.047	Mtus1	28971	0.212448998	0.045221446	0.081591051	0.056975457
chr8	23184678	23184867	190	0.044	Loxl2	59455	0.033107474	0.028156664	0.086812392	0.024788173
chr8	26429564	26429693	130	0.047	EG432870	3988	0.089238216	0.060735815	0.073336461	0.048605512
chr8	28226653	28226969	317	0.005	Zfp395	16863	0.070140437	0.097469136	0.149103273	-0.08306212
chr8	29191531	29191754	224	0.044	Dusp4	13370	0.074664184	0.033243325	0.011987359	0.148375941
chr8	30402021	30402307	287	0.011	Rbpms	123000	0.059656602	0.066092099	0.074840217	0.051525926
chr8	37384529	37384565	37	0.015	Zfp703	163716	0.056355149	0.074172948	0.025017061	0.054509768
chr8	41907137	41907600	464	0.006	Myst3	1715	0.033951722	0.049325479	0.032881859	0.092351017
chr8	43152678	43152944	267	0.012	4921537 P18Rik	2731	0.101774677	0.10468965	0.001638054	0.071895792
chr8	48921440	48921670	231	0.017	Ube2v2	343	0.549728625	0.045360426	0.723948231	0.032717176
chr8	66701734	66702089	356	0.006	Pde7a	415	0.000239452	0.133829622	0.022705523	0.060627845
chr8	70588403	70588535	133	0.047	Slco5a1	115217	0.03737239	0.118897956	0.027050638	0.095556995
chr8	72271032	72271813	782	0.005	Eyal	2632	0.006526032	0.055557647	0.039520429	0.068107877
chr8	79577640	79577971	332	0.012	3110050 N22Rik	302	0.027335434	0.120166491	0.013070318	0.060396267
chr8	86377077	86377304	228	0.012	Car2	950	0.205873288	0.079580679	0.186796984	0.039905492
chr8	1.1E+08	1.1E+08	213	0.032	Nudcd1	0	0.005606233	0.146137424	0.051823134	0.087134642
chr8	1.24E+08	1.24E+08	1123	0.005	Der1l	2649	0.016160863	0.201700733	0.089516329	0.026571651
chr8	1.26E+08	1.26E+08	254	0.017	Mtss1	82626	0.053762046	0.044152252	0.016721463	-

										0.073319669
chr8	1.26E+08	1.26E+08	670	0.005	Mtss1	63675	0.105146153	0.033045224	0.109370839	0.020581098
chr8	1.26E+08	1.26E+08	409	0.012	Sqle	227	0.018217776	0.088449027	0.056776096	0.09498824
chr8	1.29E+08	1.29E+08	500	0.026	Myc	152321	0.029892253	0.074853732	0.013289519	0.075490732
chr8	1.31E+08	1.31E+08	904	0.03	0910001 A06Rik	58431	0.006432972	0.083997065	0.053704732	-0.0768861
chr8	1.31E+08	1.31E+08	377	0.006	Ddef1	919	0.091831138	-0.05001441	0.025151831	0.103694855
chr8	1.41E+08	1.41E+08	553	0.009	18100 44A24Rik	189344	0.457540782	0.018224753	0.107424574	-0.04111049
chr8	1.45E+08	1.45E+08	378	0.012	AA409316	4016	0.026970252	-0.06895942	0.004605833	0.117723719
chr10	1402817	1403101	285	0.033	Adarb2	371069	0.008030471	0.051764156	0.093009663	0.035032577
chr10	3895156	3895674	519	0.026	Klf6	58172	0.055394379	0.036857931	0.25164663	0.019903847
chr10	5485881	5486200	320	0.012	Net1	22431	0.081087469	0.097455401	0.118015948	0.068368104
chr10	5490224	5490845	622	0.005	Net1	1444	0.149235639	0.058475426	0.216396182	0.038191461
chr10	5708419	5708659	241	0.012	Asb13	0	0.059598542	-0.04917547	0.007263468	0.084979203
chr10	6190543	6191242	700	0.006	Rbm17	54400	0.017146089	0.073584795	0.019469104	-0.07219703
chr10	21571119	21571557	439	0.005	Neb1	121815	0.30595094	0.036460642	0.299758058	0.021884691
chr10	25305780	25305987	208	0.05	Thnsl1	179	0.101278552	0.053808868	0.028015814	0.078205159
chr10	70884710	70884845	136	0.05	Vps26a	671	0.087081301	0.069417137	0.034050172	0.062072851
chr10	73300885	73301322	438	0.005	Cdh23	129697	0.080482241	0.055621735	0.089036951	0.031747595
chr10	82175262	82175938	677	0.009	5730469 M10Rik	7325	0.130456278	0.059308364	0.137804668	0.067323607
chr10	82180528	82180732	205	0.015	5730469 M10Rik	9852	0.061117646	0.080669928	0.120311064	0.081479412
chr10	82279570	82279941	372	0.028	Tspan14	60467	0.013820019	0.139269935	0.00467758	0.075130378
chr10	91006627	91007328	702	0.008	Lipa	2702	0.074112974	0.077302683	0.116122616	0.040575054

chr10	97144444	97145224	781	0.005	Sorbs1	48677	0.027219413	0.07734614	0.001956324	0.071898348	-
chr10	97517128	97517433	306	0.032	Entpd1	1149	0.026474464	0.077219508	0.061995517	0.046133335	-
chr10	98416374	98417030	657	0.006	Pik3ap1	52458	0.002273819	0.160411722	0.116710163	0.070339204	-
chr10	99160683	99160877	195	0.044	Rrp12	186	0.080496726	0.057407697	0.116057055	0.075788125	-
chr10	1.02E+08	1.02E+08	210	0.022	Scd1	1993	0.049406421	0.040765323	0.189306401	0.060816137	-
chr10	1.03E+08	1.03E+08	418	0.033	Btrc	112265	0.057694353	0.085267828	0.012106663	0.164397338	-
chr10	1.05E+08	1.05E+08	762	0.005	As3mt	1892	0.004186243	0.118357444	0.018854098	0.05787133	-
chr10	1.05E+08	1.05E+08	294	0.026	Sh3pxd2a	157260	0.000643737	0.11508831	0.000122591	0.095521028	-
chr10	1.13E+08	1.13E+08	140	0.044	Adra2a	2305	0.153991099	0.061788635	0.056353675	0.126197788	-
chr10	1.15E+08	1.15E+08	448	0.035	Tcf7l2	42599	0.00035593	0.158699963	0.353841481	0.038008249	-
chr10	1.15E+08	1.15E+08	830	0.009	Tcf7l2	76886	0.001798348	0.127830599	0.008485517	0.065095072	-
chr10	1.15E+08	1.15E+08	479	0.006	Tcf7l2	109944	0.100312432	0.057000362	0.060303239	0.086105446	-
chr10	1.16E+08	1.16E+08	806	0.009	Adrb1	21804	0.272925161	-0.0481567	0.14171411	0.042851261	-
chr10	1.18E+08	1.18E+08	295	0.006	Atrnl1	404090	0.162784849	0.055245791	0.385223126	-0.03548502	-
chr10	1.23E+08	1.23E+08	343	0.017	Brwd2	0	0.157144273	0.045241863	0.032353194	0.096461872	-
chr10	1.24E+08	1.24E+08	144	0.03	Tacc2	112343	0.042701586	0.050719844	0.010493753	0.044891116	-
chr10	1.24E+08	1.24E+08	305	0.012	Plekha1	877	0.198129182	0.050715394	0.101876959	0.053856557	-
chr10	1.29E+08	1.29E+08	217	0.022	Dock1	980	0.055414321	0.036175689	0.217399231	0.044818476	-
chr10	1.3E+08	1.3E+08	447	0.005	Ptpre	114341	0.006558867	0.157671746	0.022388868	0.093969223	-
chr10	1.32E+08	1.32E+08	487	0.006	Glrx3	155537	0.259498246	0.021822295	0.048281286	0.042428248	-
chr10	1.33E+08	1.33E+08	595	0.015	Tcerg11	182449	0.023710989	0.071039385	0.016816568	0.07245519	-
chr10	1.34E+08	1.34E+08	162	0.046	Bnip3	1862	0.032971233	0.105305104	0.022670161	-0.09346149	-
chr10	1.35E+08	1.35E+08	235	0.006	6430531 B16Rik	4090	0.04716606	0.118765257	0.02780257	0.053334838	-

chr10	1.35E+08	1.35E+08	1195	0.006	Cyp2e1	8867	0.098711704	0.049678185	0.037460112	0.078945329
chr12	1663136	1663829	694	0.005	Wnt5b	21848	0.043716238	0.046192522	0.049488495	0.070552105
chr12	6166239	6166263	25	0.011	Vwf	50822	0.248521563	0.043982022	0.022024994	0.079555472
chr12	6469500	6469539	40	0.006	Scnn1a	11615	0.03543076	0.03100671	0.071225305	0.042570688
chr12	7060921	7061200	280	0.032	Ptpn6	424	0.000293744	0.145500528	0.01348777	-0.08116907
chr12	7308164	7308415	252	0.015	Clstn3	30684	0.035942937	0.126870767	0.024351012	0.062460805
chr12	8216915	8217820	906	0.006	C3ar1	1261	0.114586135	0.033344046	0.056823806	0.080856054
chr12	8275965	8276317	353	0.026	Clec4a4	138	0.013803864	0.124748032	0.010435524	0.083632699
chr12	11812172	11812279	108	0.022	Etv6	9226	0.005150697	0.120887626	0.023111836	0.055501587
chr12	14928365	14928608	244	0.016	H2afj	872	0.115301893	0.022948594	0.007076155	0.116071913
chr12	14995468	14996149	682	0.006	Art4	197	0.047220256	0.076344607	0.103695806	0.069175548
chr12	15111717	15112453	737	0.005	Arhgdib	1519	0.00956376	0.100766318	0.029894071	0.074887817
chr12	26392741	26393036	296	0.031	Sspn	34525	0.032865677	0.114081438	0.136647608	0.074148874
chr12	40010029	40010986	958	0.005	Abcd2	2759	0.045439035	0.052089161	0.014124607	0.077337776
chr12	50419685	50419898	214	0.017	Racgap1	450	0.067364544	0.028632215	0.055732133	0.056997761
chr12	53268780	53268925	146	0.047	Krt8	26221	0.417767017	-0.0228861	0.558644222	0.044011508
chr12	53497808	53497996	189	0.044	Soat2	448	0.059851859	0.04586622	0.01856073	0.041060574
chr12	54686256	54686746	491	0.04	Nfe2	6386	0.047686589	0.052394071	0.137424206	-0.03739656
chr12	62658334	62658491	158	0.047	Usp15	3106	0.012820313	0.080748795	0.01870305	0.101326683
chr12	69140644	69140871	228	0.022	Slc35e3	687	0.00294898	-0.05840808	0.174567673	0.028840302
chr12	75874872	75875171	300	0.005	Glpr1	375	0.028932969	0.079091903	0.133593051	0.061851978
chr12	76662008	76662319	312	0.033	Osblp18	174550	0.230407077	0.084261368	0.366828779	0.047464744
chr12	94978299	94978583	285	0.03	Tmcc3	61933	0.117548072	0.021978496	0.007529745	0.075171522

chr12	95000081	95001986	1906	0.026	Tmcc3	43751	0.004387317	0.116714272	0.167215876	0.026028237
chr12	96813903	96814310	408	0.006	Pctk2	16844	0.097873514	0.084093689	0.077434262	0.102265772
chr12	1.01E+08	1.01E+08	36	0.017	Uhrflbp1l	3883	0.2054361	0.045371194	0.01143517	0.100915673
chr12	1.02E+08	1.02E+08	285	0.011	Slc5a8	59109	0.068941654	0.057969332	0.047063901	0.048213168
chr12	1.02E+08	1.02E+08	1912	0.005	Chpt1	1466	0.086619668	0.103113517	0.219034114	0.08831166
chr12	1.03E+08	1.03E+08	629	0.005	Pmch	33525	0.004350063	0.115790802	0.264567514	0.049532817
chr12	1.04E+08	1.04E+08	221	0.017	Tdg	752	0.005057558	0.136091463	0.056719502	0.035545438
chr12	1.07E+08	1.07E+08	127	0.026	Ckap4	16968	0.036423307	0.046232273	0.03125838	0.116741557
chr12	1.07E+08	1.07E+08	236	0.022	Rfx4	55034	0.452216789	0.039977643	0.661016157	0.018901322
chr12	1.09E+08	1.09E+08	164	0.049	Ssh1	41530	0.017916085	0.035229392	0.057734338	0.057221546
chr12	1.1E+08	1.1E+08	453	0.005	Kctd10	56723	0.495308113	0.036933183	0.107609453	0.050279414
chr12	1.1E+08	1.1E+08	318	0.012	Git2	478	0.240157029	0.050905326	0.030801005	0.073031388
chr12	1.15E+08	1.15E+08	291	0.033	Tbx3	12908	0.039598932	0.062105131	0.080103137	0.058777531
chr12	1.15E+08	1.15E+08	197	0.017	Tbx3	3086	0.00275724	-0.14756063	0.001537324	0.068605604
chr12	1.15E+08	1.15E+08	867	0.005	Tbx3	6515	0.00030568	0.187331894	0.001286375	0.141162287
chr12	1.17E+08	1.17E+08	21	0.05	2410131 K14Rik	1337	0.095202773	0.072915207	0.428718333	0.022901692
chr12	1.17E+08	1.17E+08	1006	0.006	Fbxw8	65405	0.047336201	-0.0429966	0.007012339	0.094022658
chr12	1.19E+08	1.19E+08	343	0.012	Taok3	543	0.030025023	0.061604516	0.024758284	0.060254613
chr12	1.22E+08	1.22E+08	630	0.035	Tmem120b	33014	0.013695007	0.095305859	0.096425055	0.061082698
chr12	1.25E+08	1.25E+08	470	0.006	Ncor2	126377	0.102138472	0.049625685	0.098288525	0.072071702
chr12	1.25E+08	1.25E+08	146	0.009	Scarb1	14604	0.171709363	0.077782925	0.354573505	0.037757024
chr12	1.26E+08	1.26E+08	234	0.035	Aacs	19023	0.115106488	0.074375332	0.004487949	0.068974009
chr22	18298189	18298467	279	0.012	Bid	39778	0.036134946	0.088448521	0.013570732	0.072441841
chr22	24761416	24761463	48	0.017	Specc1l	45885	0.344188283	0.014164191	0.067092193	0.044626658
chr22	30278371	30278842	472	0.042	Mtmt3	300	0.162418389	0.047787343	0.329332465	0.045700304
chr22	36924935	36925290	356	0.012	Eif3d	0	0.033885498	0.073884507	0.106029245	0.058064788

chr22	38472613	38473870	1258	0.005	Slc16a8	3862	0.03773117	0.050802924	0.050792568	0.05132624
chr22	40858582	40860665	2084	0.005	Mkl1	58828	0.001384889	0.125891594	0.019491324	0.111849464
chr22	43555410	43557147	1738	0.006	Tspo	8007	0.018177759	0.050771113	0.173972034	0.035722293
chr22	45561031	45561107	77	0.044	Nup50	708	0.264933651	0.033646427	0.020614385	0.09905398
chr22	50955702	50956285	584	0.005	Ncaph2	7477	0.069548977	0.071080994	0.058356976	0.041453832
chr17	1220102	1220359	258	0.005	Tusc5	32046	0.00498105	0.124949686	0.331277878	0.037317959
chr17	7483300	7483645	346	0.029	Cd68	288	0.004383267	0.086940803	0.004551923	0.051619704
chr17	8027102	8027420	319	0.029	Hes7	4	0.039741912	0.033411302	0.158273454	-0.02601069
chr17	8867824	8868379	556	0.005	Pik3r5	611	0.128040105	0.057670787	0.458486372	0.038137455
chr17	9950908	9951126	219	0.028	Gas7	46063	0.208451228	0.047643312	0.065000979	0.064380479
chr17	26646057	26646266	210	0.026	Tmem97	0	0.009588159	0.219201828	0.041101816	0.168257023
chr17	29150010	29150903	894	0.006	Crlf3	749	0.064732777	0.026243112	0.054155807	0.070975564
chr17	29641021	29641319	299	0.015	Evi2b	0	0.137955415	0.060964811	0.009073702	0.103736739
chr17	34415897	34416118	222	0.026	Ccl3	1024	0.001156209	0.090858161	0.043889966	0.052169682
chr17	34417557	34418034	478	0.009	Ccl3	51	0.001156209	0.090858161	0.043889966	0.052169682
chr17	35299693	35299863	171	0.022	Lhx1	5283	0.353425615	0.019486541	0.080261005	0.03668788
chr17	40142289	40142472	184	0.032	Dnajc7	27020	0.07140143	0.050557861	0.179317913	-0.0406226
chr17	45908521	45908890	370	0.026	Mrpl10	3	0.158883651	0.046498288	0.152146362	0.048085714
chr17	47819090	47819795	706	0.005	5730593 F17Rik	17777	0.007399875	0.102310745	0.007408441	0.126769783
chr17	47863126	47864532	1407	0.022	Myst2	1764	0.071136849	0.073246062	0.028307361	0.092132287
chr17	48940751	48941296	546	0.006	Tob1	2269	0.06118011	0.085294662	0.175848717	0.071444824
chr17	56124678	56125091	414	0.005	Sfrs1	19470	0.022776363	0.076070557	0.129254578	0.061583356
chr17	56355449	56355594	146	0.044	Mpo	3658	0.038596305	0.060609612	0.084255863	-0.04298877

chr17	56490498	56491520	1023	0.005	Hsf5	49038	0.022309925	0.109264262	0.047502101	-
chr17	62204776	62206576	1801	0.006	Ern1	891	0.076370686	0.096781936	0.146030662	0.057706948
chr17	63541217	63541473	257	0.032	Axin2	15769	0.020481163	0.049730423	0.106289649	-
chr17	64448688	64449382	695	0.005	Prkca	120673	0.14072088	0.047904076	0.155558681	-
chr17	66877696	66877766	71	0.044	Abca8a	53490	0.122227015	0.086695187	0.015035725	0.050854997
chr17	73406576	73407600	1025	0.006	Grb2	4125	0.110354272	0.048225572	0.011694515	0.073869847
chr17	73630171	73630823	653	0.026	Recql5	32121	0.006844407	0.104660956	0.002223662	0.207049308
chr17	76375529	76375618	90	0.047	Pgs1	567	0.160821825	0.071059014	0.26928162	0.039626158
chr17	79419543	79420749	1207	0.006	Bahcc1	705	0.017498542	0.105982501	0.024719301	0.042729251
chr17	80050393	80050984	592	0.017	Fasn	4126	0.035750412	0.102212516	0.002367071	0.106729786
chr17	80052124	80053059	936	0.009	Fasn	2706	0.00234477	0.110938993	0.045378089	0.090742194
chr17	80416305	80416449	145	0.044	Narf	171	0.377968144	0.039260145	0.613152845	-
chr5	10304741	10305342	602	0.009	Cmb1	2007	0.016968763	0.102322221	0.044958962	-
chr5	32780268	32780484	217	0.043	Npr3	53629	0.043125176	0.062892296	0.017355784	-
chr5	34655547	34655935	389	0.029	Rai14	1628	0.04433336	0.097710785	0.057769423	0.068167382
chr5	34687673	34687965	293	0.029	Rai14	23832	0.027202557	0.113639208	0.000316782	0.222487047
chr5	36535659	36536198	540	0.009	Slc1a3	70605	0.188674087	0.035795174	0.154389728	-
chr5	55737286	55737497	212	0.033	Ankrd55	157337	0.016894229	0.094145368	0.097086997	0.041114561
chr5	64333579	64333820	242	0.022	Sdccag10	205522	0.011515023	0.080503852	0.221219504	0.036053507
chr5	66491448	66492361	914	0.005	Cd180	251	0.010515394	0.065840584	0.032887588	-
chr5	67585536	67585875	340	0.033	Pik3r1	74846	0.027604432	0.069279842	0.05005877	-
chr5	74244430	74245271	842	0.005	1700029 F12Rik	50707	0.051883314	0.077775325	0.163557371	-
chr5	77803248	77803367	120	0.03	Lhfpl2	118687	0.014339375	0.094089602	0.000121396	-

chr5	79028795	79029354	560	0.032	Papd4	98240	0.404746582	0.014708512	0.047724769	0.082858532
chr5	1.07E+08	1.07E+08	257	0.011	Efna5	96616	0.040259849	0.089666375	0.005299128	0.066779882
chr5	1.07E+08	1.07E+08	438	0.006	Efna5	64136	0.103233424	0.063036468	0.206316816	0.019221009
chr5	1.08E+08	1.08E+08	299	0.011	Fert2	30883	0.014590337	0.144368703	0.032578258	0.065941939
chr5	1.34E+08	1.34E+08	288	0.022	Sar1b	0	0.19291661	0.155371226	0.336384668	0.016634489
chr5	1.35E+08	1.35E+08	229	0.047	BC027057	341	0.121578462	-0.04209435	0.165450178	0.035528722
chr5	1.42E+08	1.42E+08	525	0.006	Spry4	26861	0.093448335	0.087272102	0.312019227	0.054293185
chr5	1.48E+08	1.48E+08	406	0.006	Adrb2	2176	0.005235308	0.099159277	0.055307519	0.100396049
chr5	1.49E+08	1.49E+08	251	0.012	Ppargc1b	535	0.070181892	0.0958248	0.122617345	0.028066474
chr5	1.49E+08	1.49E+08	691	0.006	Csflr	714	0.008300488	0.087832678	0.011173575	0.049165072
chr5	1.51E+08	1.51E+08	473	0.024	Slc36a2	108	0.333779062	0.04041369	0.020229623	0.057497869
chr5	1.7E+08	1.7E+08	263	0.011	Kcnmb1	45521	1.42E-05	0.194462311	0.00669345	0.100721483
chr5	1.76E+08	1.76E+08	688	0.012	Hk3	182	0.178871723	0.050743362	0.233169223	0.039251216
chr5	1.79E+08	1.79E+08	164	0.012	Adams2	35826	0.010600527	0.111699146	0.004506479	0.059363616
chr7	1408433	1408951	519	0.006	Micall2	64015	0.01030415	0.063819595	0.002222894	0.199369417
chr7	8302335	8302647	313	0.012	Ical	149	0.852262755	0.008577271	0.210938579	0.051894475
chr7	26460385	26460633	249	0.015	Snx10	91887	0.017227015	0.112177321	0.020509591	0.104041417
chr7	27201206	27201499	294	0.027	Hoxa9	4830	0.000244	0.154545194	0.000896047	0.131389066
chr7	27202553	27202993	441	0.044	Hoxa9	3340	0.00772616	0.163900092	0.098968687	0.037616491
chr7	27203073	27203466	394	0.008	Hoxa9	2880	0.00772616	0.163900092	0.098968687	0.037616491
chr7	30287772	30288094	323	0.009	Znrf2	38759	0.502192871	0.028442713	0.197857941	0.076881409
chr7	47672033	47673452	1420	0.006	Tns3	24418	0.001650023	0.090694865	0.01767947	0.067112733
chr7	50345715	50346835	1121	0.005	Ikzf1	1486	0.000203584	0.123971624	0.000648778	0.128215817
chr7	73624879	73625346	468	0.005	Lat2	610	0.025835427	-	0.073432421	0.07910527

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chr7	75549990	75550275	286	0.03	Por	6204	0.240896483	0.019749676	0.228422084	0.026925827
chr7	75957929	75958231	303	0.012	Ywhag	24237	0.014476618	0.079952002	0.001556626	0.148300136
chr7	76828348	76828576	229	0.042	Fgl2	528	0.032838026	0.069069628	0.072158041	0.095986623
chr7	97500452	97501026	575	0.005	Asns	637	0.05303718	0.096679458	0.11633825	0.047365147
chr7	1E+08	1E+08	283	0.012	Trfr2	1124	0.074148968	0.074548168	0.048991726	0.082537723
chr7	1.05E+08	1.05E+08	217	0.03	Atxn7l1	167219	0.001087181	0.126246501	0.023129641	0.102921606
chr7	1.07E+08	1.07E+08	526	0.005	Prkar2b	15286	0.001244862	0.088189068	0.052518879	0.057034208
chr7	1.08E+08	1.08E+08	786	0.006	Lamb1-1	27653	0.256006703	0.064722568	0.289143787	0.05643269
chr7	1.08E+08	1.08E+08	362	0.005	Lamb1-1	51183	0.020384645	0.090978916	0.010390466	0.114733938
chr7	1.28E+08	1.28E+08	988	0.005	Lep	27192	0.193642885	0.043408718	0.372316108	0.037308041
chr7	1.28E+08	1.28E+08	209	0.042	Lep	6068	0.177358186	0.052936735	0.08475912	0.050701004
chr7	1.31E+08	1.31E+08	528	0.043	Tsgal3	193618	0.062198878	0.062047453	0.124462472	0.066968759
chr7	1.31E+08	1.31E+08	306	0.012	Podxl	350	0.099786111	0.055238515	0.09342786	0.08650265
chr7	1.4E+08	1.4E+08	1373	0.005	Tbxas1	36	7.05E-05	0.203984492	0.012069378	0.094241458
chr7	1.4E+08	1.4E+08	465	0.009	Slc37a3	30231	0.219770505	0.023951494	0.103260316	0.079376392
chr7	1.51E+08	1.51E+08	177	0.035	Nub1	553	0.00124038	0.194317077	0.000247585	0.144171977
chr14	20898926	20899114	189	0.012	Osgep	31189	0.001031265	0.133171333	0.005996648	0.109023293
chr14	21568858	21569390	533	0.035	Zfp219	3747	0.227490337	0.046680964	0.083284561	0.049659277
chr14	23788798	23789618	821	0.005	Pabpn1	901	0.113051505	0.098799804	0.014235179	0.070986247
chr14	31888216	31888303	88	0.026	Heatr5a	1354	0.051428286	0.077015105	0.074113648	0.052222637
chr14	39737165	39737329	165	0.046	Ctage5	2120	0.091711178	0.047880934	0.083113321	0.061483438
chr14	55833724	55834573	850	0.012	D14 Ertd436e	26708	0.128314832	0.019852413	0.062240794	0.035332666
chr14	55834889	55835484	596	0.033	D14 Ertd436e	26166	0.128314832	0.019852413	0.062240794	0.035332666

chr14	74961318	74961491	174	0.022	Npc2	1191	0.02971491	0.068992065	0.174558788	0.037000804
chr14	75361059	75361571	513	0.033	Dlst	12923	0.002412449	0.165986778	0.006711909	0.148309561
chr14	77791405	77791671	267	0.011	Gstz1	10333	0.010968896	0.134177876	0.049884578	0.089298147
chr14	81867674	81868031	358	0.015	Ston2	37969	0.081282534	0.073907314	0.268537265	0.049810371
chr14	89707672	89708616	945	0.009	Foxn3	192777	0.005943992	0.082570716	0.097830571	0.051674454
chr14	93117257	93117768	512	0.029	Rin3	84784	0.003786645	0.112199089	0.01225759	0.071423943
chr14	97180040	97180391	352	0.006	Vrk1	72542	0.161683822	0.025082244	0.038464686	0.048180668
chr14	1.01E+08	1.01E+08	749	0.006	Evl	81191	0.003391464	0.113839928	0.004730441	0.106016745
chr9	14203697	14204081	385	0.005	Nfib	100379	0.001304379	0.122130904	0.009561952	0.121569828
chr9	32457089	32457364	276	0.005	Ddx58	33770	0.019332685	0.140039676	0.303952013	-0.02997273
chr9	36778847	36778989	143	0.033	Pax5	252932	0.017411606	0.117459817	0.022346231	0.106487141
chr9	93565879	93566460	582	0.006	Syk	11788	0.007420128	0.094453504	1.49E-05	0.197044812
chr9	95726604	95726801	198	0.03	Fgd3	0	0.000782954	0.190154032	0.106857929	0.053373774
chr9	1.13E+08	1.13E+08	387	0.009	Txn1	2744	0.153227162	0.059319005	0.063978233	0.069727536
chr9	1.13E+08	1.13E+08	479	0.005	Svep1	28635	0.635801168	0.026481336	0.32169114	0.045576333
chr9	1.14E+08	1.14E+08	387	0.006	Gng10	615	0.008616226	0.054665936	0.004046392	0.113837927
chr9	1.16E+08	1.16E+08	318	0.033	Rgs3	108843	0.034041424	0.053038017	0.076859759	0.032379635
chr9	1.17E+08	1.17E+08	322	0.045	Orm1	0	0.089241078	0.033000789	0.173649309	0.031700782
chr9	1.17E+08	1.17E+08	468	0.006	Akna	2023	0.090278173	0.057770456	0.064277099	0.083163963
chr9	1.18E+08	1.18E+08	333	0.016	Tnfsf8	0	0.000933629	0.161745875	0.010526571	0.109511716
chr9	1.3E+08	1.3E+08	369	0.006	9130404 D14Rik	41455	0.121434378	-0.03946649	0.006459746	0.116243706
chr9	1.31E+08	1.31E+08	610	0.005	Spna2	45170	0.021422761	0.053623327	0.017081786	0.090287465
chr9	1.33E+08	1.33E+08	208	0.03	Fbnp1	1050	0.060174876	0.083590765	0.05641399	0.093572322
chr13	29293918	29294030	113	0.032	Slc46a3	979	0.234074293	0.075604237	0.130567277	0.047285533

chr13	32780792	32781098	307	0.012	Brca2	112608	0.477862977	0.019990114	0.189502768	-0.03662977
chr13	36049213	36050887	1675	0.005	Mab2111	0	0.000513404	0.083527653	0.000333282	0.083898174
chr13	40809925	40810619	695	0.009	Foxo1	332765	0.449595212	0.027281116	0.026363464	0.067673717
chr13	40943529	40943850	322	0.006	Foxo1	248828	0.045248093	0.073827692	0.097759862	0.042986649
chr13	44936911	44937231	321	0.012	Tsc22d1	64627	0.071609449	0.039506342	0.022718659	0.050643262
chr13	45564095	45564224	130	0.026	12000 11118Rik	323	0.004529161	0.179434382	0.048036648	0.105042022
chr13	45694501	45694858	358	0.006	Gtf2f2	0	0.004084029	0.071848765	0.04120081	0.076936457
chr13	46751279	46752608	1330	0.005	Lcpl	44287	0.014544051	0.091356551	0.02164575	0.098119319
chr13	46753288	46754147	860	0.009	Lcpl	42705	0.014544051	0.091356551	0.02164575	0.098119319
chr13	49683338	49683473	136	0.044	Fndc3a	105975	0.149221767	0.029842321	0.20143602	0.044226379
chr13	50204794	50205145	352	0.022	Arl11	1201	0.044279538	0.107739618	0.046926469	0.048619668
chr13	1.14E+08	1.14E+08	171	0.022	Grtp1	347	0.229393431	0.053562829	0.153692182	0.096703406
chr3	9911613	9911971	359	0.029	Cidec	7236	0.014015847	0.066336204	0.028881232	0.05617051
chr3	15667550	15667785	236	0.011	Btd	13549	0.057143936	0.043614963	0.01757608	0.042154639
chr3	15681784	15682347	564	0.006	Btd	23503	0.152744834	-0.05127628	0.091948578	0.056366809
chr3	24869934	24870287	354	0.008	Rarb	598099	0.029777881	0.046387087	0.111070939	0.109112789
chr3	32412954	32413251	298	0.006	Cmtm8	56143	0.172866571	0.03624256	0.165838952	0.039884397
chr3	37907270	37907753	484	0.005	Ctdspl	4077	0.374453931	0.023449625	0.113248746	0.053198313
chr3	38007994	38008396	403	0.009	Ctdspl	102518	0.031627285	0.085747522	0.080380047	0.058105565
chr3	40499457	40499546	90	0.05	Rpl14	656	0.026209488	0.061704304	0.17181901	-0.03784743
chr3	46036856	46037289	434	0.011	Fyco1	42	0.013540094	0.234110654	0.107252986	0.152537306
chr3	52445047	52445468	422	0.006	Phf7	319	0.164356522	0.045244721	0.118265149	0.022978991
chr3	52567262	52567405	144	0.042	Nt5dc2	3223	0.130156467	0.037378327	0.009817152	0.115683951
chr3	57262305	57262906	602	0.006	Appl1	461	0.239989212	-	0.334632607	0.030190953

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chr3	65434405	65434559	155	0.046	Magi1	143197	0.049562076	0.048552937	0.136872297	0.047379194
chr3	66633695	66634011	317	0.032	Lrig1	61174	0.087614339	0.038846936	0.028552309	0.066755725
chr3	67049442	67049635	194	0.033	Kbtbd8	655	0.159046189	0.057621246	0.054193913	0.06292396
chr3	72870608	72870903	296	0.036	Shq1	25788	0.026213972	0.070105607	0.020065667	0.074226033
chr3	72896699	72897027	329	0.047	Shq1	466	0.004251486	0.192497184	0.034505003	0.046382452
chr3	1.13E+08	1.13E+08	1126	0.005	Cd200r1	617	0.009566716	0.067486844	0.011117094	0.060831706
chr3	1.13E+08	1.13E+08	568	0.008	Boc	31960	0.026237876	0.087180801	0.04824537	0.060003923
chr3	1.24E+08	1.24E+08	224	0.015	Umps	89428	0.053370691	0.053070703	0.081051452	0.058941427
chr3	1.25E+08	1.25E+08	235	0.033	Itgb5	92104	0.112577227	0.052593515	0.011370824	0.112851305
chr3	1.25E+08	1.25E+08	328	0.028	Itgb5	91521	0.112577227	0.052593515	0.011370824	0.112851305
chr3	1.25E+08	1.25E+08	206	0.033	Itgb5	55665	0.33380135	0.051607354	0.214351388	0.046073299
chr3	1.28E+08	1.28E+08	409	0.026	Mgll	29843	0.300572926	0.046883681	0.011379527	-0.05814309
chr3	1.34E+08	1.34E+08	358	0.015	Rab6b	767	0.012336117	0.053271596	0.021698788	-0.10620353
chr3	1.38E+08	1.38E+08	24	0.05	Cep70	781	0.091346454	0.055603918	0.134506833	0.020105915
chr3	1.41E+08	1.41E+08	1142	0.005	Zbtb38	2118	0.01672593	0.121198507	0.065315651	-0.09495155
chr3	1.5E+08	1.5E+08	360	0.012	2810407 C02Rik	685	0.000503571	-0.14397228	0.000438028	0.109932805
chr3	1.51E+08	1.51E+08	283	0.012	P2ry14	182	0.115599	0.047542803	0.226721227	0.036047052
chr3	1.51E+08	1.51E+08	235	0.033	P2ry12	44	0.314598291	0.057538343	0.068461354	0.089714333
chr3	1.57E+08	1.57E+08	421	0.006	Veph1	104131	0.000152002	0.188356272	0.004779003	0.130964675
chr3	1.6E+08	1.6E+08	322	0.024	Il12a	917	0.510455058	0.037543221	0.26771562	0.062963536
chr3	1.7E+08	1.7E+08	151	0.033	Phc3	1137	0.06071042	0.081619325	0.290326978	0.020625775
chr3	1.71E+08	1.71E+08	233	0.033	Slc2a2	142633	0.246755268	0.062234751	0.37095251	0.043315768
chr3	1.85E+08	1.85E+08	307	0.033	Liph	39515	0.11891308	0.025769524	0.062454307	0.078541633

chr3	1.87E+08	1.87E+08	272	0.005	Eif4a2	559	0.057501757	0.053855992	0.00334395	-
chr3	1.87E+08	1.87E+08	352	0.005	Bcl6	24573	0.183180484	0.023522408	0.061134987	-
chr3	1.89E+08	1.89E+08	656	0.006	Lpp	579307	0.255225767	0.039438945	0.250766738	-0.03871692
chr3	1.96E+08	1.96E+08	1239	0.032	Tctex1d2	18267	0.200280698	0.077940287	0.14598354	-
chr3	1.96E+08	1.96E+08	1257	0.005	Fbxo45	8610	0.051206131	0.032303493	0.076985143	-
chr16	16123803	16124667	865	0.005	Abcc1	39809	0.028137766	0.062463282	0.092616577	0.040749733
chr16	20909935	20910137	203	0.015	Dcun1d3	1217	0.085974868	0.056191121	0.030928297	0.107792016
chr16	30455438	30455975	538	0.005	Sephs2	1221	0.03253984	-0.0577708	0.041044579	-
chr16	46957730	46958679	950	0.005	Gpt2	28479	0.031950761	0.034656493	0.077947645	-
chr16	50059655	50059880	226	0.047	Tmem188	410	0.063328638	0.103644757	0.056521478	-
chr16	50715116	50715299	184	0.011	9130017 C17Rik	0	0.000204672	0.169680371	0.012381409	-
chr16	55540360	55540833	474	0.006	Mmp2	26328	0.012314177	0.055940936	0.083100844	-
chr16	56137894	56138256	363	0.006	Gnao1	85203	0.004769343	0.066758804	0.003215157	-
chr16	57278607	57278995	389	0.022	Arl2bp	161	0.317131448	0.045084544	0.226855795	-
chr16	69457347	69457490	144	0.044	Cyb5b	632	0.01244399	0.086794906	0.184650573	-
chr16	79370715	79370926	212	0.024	Wwox	1022931	0.118336141	0.035616683	0.098991552	-
chr16	83549673	83549934	262	0.044	Cdh13	837835	0.092723296	0.034427003	0.079016286	-
chr16	85376056	85376680	625	0.005	Gse1	144355	0.182709459	0.023703324	0.056581861	-
chr16	87903345	87904117	773	0.006	BC048644	147	0.034324129	0.089302202	0.034224962	-
chr16	88770501	88770848	348	0.012	Rnfl66	1390	0.043240371	0.073898435	0.137684355	-
chr21	16057979	16058284	306	0.011	Samsn1	144360	0.414561669	0.033457948	0.237872414	-
chr21	33689979	33690246	268	0.033	Mrap	16472	0.149919203	0.039646958	0.115863135	-
chr21	35448249	35449067	819	0.005	Slc5a3	1968	0.003752114	0.121181521	0.006888168	-

chr21	36398548	36398828	281	0.011	Runx1	24726	0.000151796	-0.15178274	0.001644441	0.119934451
chr21	36420456	36421805	1350	0.005	Runx1	0	4.31E-06	0.279855942	0.00055991	0.176657529
chr21	37412563	37413036	474	0.006	Setd4	17406	0.057139401	0.073919244	0.38706981	0.029344222
chr21	39868683	39869555	873	0.01	Erg	71852	0.000107641	0.134357154	0.004244138	0.091090816
chr21	40776034	40777497	1464	0.005	Lcca5l	33478	0.304302694	0.020796314	0.033232229	0.047454765
chr18	2960647	2960946	300	0.03	Lpin2	10199	0.226393124	0.074511207	0.221852968	0.065237008
chr18	10457031	10457507	477	0.006	Apcddl	2177	0.093670381	0.042061125	0.13835125	0.026116591
chr18	46277273	46277494	222	0.012	Gm672	169837	0.060855394	0.080607141	0.002350005	0.17815372
chr18	56809110	56809370	261	0.017	Sec11c	1867	0.047576474	0.102872363	0.081715262	0.086228022
chr18	77440474	77440960	487	0.006	Ctdpl	628	0.124164823	-0.03050826	0.022559577	0.103251482
chr19	7733678	7734230	553	0.026	Retn	0	0.017884245	0.067224475	0.127563827	0.037882218
chr19	12920962	12921262	301	0.026	Rnaseh2a	5983	0.070335289	0.037572965	0.02018467	0.043792759
chr19	15574480	15574831	352	0.046	A430107 D22Rik	535	0.019917347	0.119050573	0.084596055	0.041823459
chr19	17669572	17669647	76	0.017	Glt25d1	751	0.503151826	0.027583264	0.401626335	0.039375052
chr19	18508116	18508457	342	0.042	Lrrc25	57	0.155971856	-0.0272848	0.017410174	0.051164222
chr19	18682314	18682390	77	0.022	Uba52	309	0.137872609	0.056746254	0.238786636	0.062029191
chr19	40750567	40750963	397	0.015	Akt2	21904	0.00536166	0.105072993	0.026742224	0.064631594
chr19	42830523	42830820	298	0.017	Tmem145	11527	0.017467435	0.125098373	0.016096425	0.174781541
chr19	42912089	42912557	469	0.026	Lipe	4500	0.063280265	0.066215746	0.080255544	0.04966715
chr19	45225646	45225969	324	0.012	Ceacam16	16447	0.096953319	0.111422729	0.082741162	0.044333143
chr19	49841249	49841895	647	0.009	Cd37	2686	0.027967301	0.091393575	0.000388475	0.135513451
chr19	51645546	51714605	69060	0.006	Cd33	0	0.026484673	0.072463543	0.042378002	0.037094866
chr19	54876429	54876765	337	0.03	Lair1	0	0.026489679	0.097348731	0.000733429	0.144786239
chr11	6267881	6268720	840	0.012	Cnga4	4830	0.001427368	0.142949402	0.095175091	0.049578861

chr11	10316893	10317243	351	0.035	Adm	11261	0.015285684	-	0.079842283	-
chr11	10324978	10325218	241	0.033	Adm	1249	0.006833463	-	0.076195352	-
chr11	13359375	13359540	166	0.042	Arntl	52507	0.287397995	0.033110364	0.151330045	0.046863197
chr11	14563296	14563419	124	0.042	Psmal1	16958	0.268961707	0.042292388	0.098957367	-
chr11	18127092	18127637	546	0.011	Saa3	5006	0.22057406	0.022446951	0.1205803	0.031998649
chr11	27490752	27491523	772	0.01	Lgr4	2692	0.020887869	-	0.168418694	-
chr11	32852321	32852814	494	0.009	Prrg4	759	0.181458128	0.058426216	0.201958387	0.043459733
chr11	47291136	47291346	211	0.026	Madd	246	0.117725556	-0.06055027	0.274985803	0.058660731
chr11	47415025	47415403	379	0.006	Sfpi1	12317	0.005612808	-0.09283909	0.020093253	-
chr11	57067776	57068975	1200	0.005	Tnks1bp1	20866	0.031735717	-	0.108159774	-
chr11	63528336	63528808	473	0.006	27000 81O15Rik	4270	0.009374816	0.075120856	0.019783585	0.057464615
chr11	64544695	64545147	453	0.012	Sfl	988	0.074007346	0.080573957	0.05951643	-
chr11	66673568	66673798	231	0.043	Pcx	24624	0.049344564	0.089939048	0.126682615	0.044461048
chr11	67036383	67036711	329	0.029	Adrbk1	2011	0.000861836	-	0.127008445	-
chr11	67165584	67166226	643	0.009	Ppplca	2700	0.038535186	0.032864959	0.003821021	0.093715644
chr11	73692113	73692198	86	0.028	Ucp2	1517	0.048032778	-	0.086506376	-
chr11	76337568	76338271	704	0.006	2210018 M11Rik	121609	0.052017168	-	0.077393197	-
chr11	1.18E+08	1.18E+08	245	0.022	Il10ra	1315	0.000608402	-	0.109964715	-
chr11	1.21E+08	1.21E+08	217	0.033	Tecta	14379	0.070233309	-	0.032303992	-
chr11	1.23E+08	1.23E+08	571	0.015	Bsx	2351	0.034711309	-	0.095100232	-
chr15	37173455	37174113	659	0.026	Meis2	210978	0.028601312	-	0.075724131	-
chr15	52404555	52404986	432	0.022	Bcl2l10	0	0.115604037	0.071796217	0.352538451	0.03906306
chr15	52820075	52820295	221	0.022	Myo5a	914	0.694329386	0.024475175	0.302911419	-

chr15	61136559	61136807	249	0.024	Rora	381025	0.001200488	0.123028637	0.091815399	0.061985343
chr15	62343394	62344034	641	0.005	Vps13c	7451	0.214289764	0.064912798	0.02432572	-0.11424508
chr15	63571085	63571484	400	0.006	Aph1c	1160	0.284299059	0.051881135	0.009652533	0.07466307
chr15	69426262	69426542	281	0.035	Glce	82581	0.018055549	0.112554107	0.053930571	0.090359063
chr15	69708054	69708318	265	0.036	Kif23	1052	0.020715438	0.049924329	0.000803319	0.072074773
chr15	77478505	77479038	534	0.033	C230081 A13Rik	153180	0.231666602	0.070100851	0.018574335	0.089551919
chr15	79471213	79471368	156	0.05	Rasgrf1	77403	0.074622439	0.035916955	0.20567207	0.042072017
chr15	89977287	89977470	184	0.044	Rhcg	47905	0.00877423	0.073352554	0.185544683	0.080818625
chr15	90701174	90701775	602	0.009	Sema4b	29547	0.085843566	0.0589646	0.10894982	0.052268715
chr15	96902600	96903113	514	0.005	Nr2f2	27667	4.02E-05	-0.13012121	0.001088048	0.175874276
chr15	96905568	96906197	630	0.006	Nr2f2	30696	0.000917372	0.151366539	7.85E-06	0.184271449
chr15	96907528	96907914	387	0.022	Nr2f2	32822	0.000917372	0.151366539	7.85E-06	0.184271449
chr20	1879865	1880324	460	0.005	Sirpa	3655	0.001414611	0.163898814	0.28961536	0.043137468
chr20	3527981	3528426	446	0.036	Atrn	39569	0.147326039	0.084492638	0.260585943	0.054837666
chr20	3746584	3746692	109	0.011	1700037 H04Rik	1278	0.139490039	0.044307576	0.18975352	0.035203827
chr20	18269464	18269677	214	0.029	6330439 K17Rik	67696	0.004138053	0.11442861	0.002225491	0.05937174
chr20	25033629	25034262	634	0.005	Acss1	4489	0.035246291	0.053724766	0.043383222	0.079822794
chr20	25251494	25252151	658	0.005	Pygb	21579	0.00912653	0.050518875	0.014407649	0.040195217
chr20	30258398	30258882	485	0.009	Bcl2l1	45565	0.062783463	0.088332976	0.038981244	0.084371149
chr20	33466246	33466396	151	0.015	Acss2	1292	0.135099492	0.049631059	0.388269862	0.026833312
chr20	35233479	35233726	248	0.012	1110008 F13Rik	517	0.178100009	0.054084787	0.462149615	0.033702996
chr20	36104587	36105045	459	0.005	Blcap	41702	0.155524754	0.044843164	0.167318597	0.047607368

chr20	36823215	36823538	324	0.026	D630003 M21Rik	54558	0.060042606	- 0.026596859	0.035622337	- 0.035290347
chr20	39314354	39315324	971	0.006	Mafb	2438	0.016335516	- 0.073400089	0.014954149	- 0.068027443
chr20	43953416	43953593	178	0.026	Sdc4	19786	0.222068845	- 0.027653814	0.067644188	- -0.0556007
chr20	47803610	47804113	504	0.006	Stau1	669	0.04857818	- 0.099555153	0.316221226	- 0.052500125
chr20	47839439	47839685	247	0.012	Ddx27	4251	0.156286748	- -0.02991861	0.047684049	- 0.043391968
chr20	48924232	48924768	537	0.022	Cebpb	87922	0.004243497	0.096121482	0.078455137	0.076853806
chr20	56135219	56135721	503	0.006	Pck1	452	0.008351783	0.073282322	0.046530677	- 0.048857478
chr20	56135935	56137320	1386	0.005	Pck1	0	0.003187762	0.087199606	0.040124916	0.040920345
chr20	56138648	56138937	290	0.016	Pck1	2893	0.003187762	0.087199606	0.040124916	0.040920345
chr20	56139464	56139982	519	0.009	Pck1	4078	0.003187762	0.087199606	0.040124916	0.040920345
chr20	58512538	58512948	411	0.03	Ppp1r3d	2200	0.099138641	0.056386238	0.054855161	0.084754804
chr4	3295253	3295496	244	0.022	Rgs12	379	0.21813933	0.078202348	0.090432956	0.036055255
chr4	38858508	38859749	1242	0.008	Tlr6	90	0.003242039	- 0.125613648	0.050924237	- 0.072456987
chr4	39408228	39410160	1933	0.005	Klb	0	0.044594542	- 0.072267154	0.237736274	- 0.045043502
chr4	84034389	84034401	13	0.015	Plac8	1423	0.000415556	- 0.184889567	0.009648694	- 0.115334801
chr4	86398976	86399113	138	0.047	Arhgap24	2326	0.063282407	0.060784671	0.005230217	0.106808245
chr4	87672032	87672388	357	0.006	Ptpn13	116187	0.100709592	- 0.079778073	0.040493552	- 0.100648092
chr4	1.09E+08	1.09E+08	352	0.005	Papss1	66631	0.052559898	- 0.072581024	0.041710937	- 0.091658981
chr4	1.09E+08	1.09E+08	207	0.024	Hadh	18992	0.04453389	0.055177969	0.171434844	0.022386383
chr4	1.14E+08	1.14E+08	773	0.005	Larp7	232193	0.027308541	- 0.078932185	0.053867885	- 0.064062514
chr4	1.2E+08	1.2E+08	206	0.005	Sec24d	78238	0.1507209	0.081634037	0.281930451	0.044129842
chr4	1.21E+08	1.21E+08	181	0.046	Pde5a	1646	0.051707622	0.108026119	0.296110272	0.057968662
chr4	1.29E+08	1.29E+08	301	0.012	Larp2	107364	0.046803004	- 0.037213593	0.050041702	- 0.080257748
chr4	1.29E+08	1.29E+08	1867	0.005	Larp2	151187	0.145876681	0.034008826	0.114284609	0.061117539
chr4	1.84E+08	1.84E+08	553	0.006	Cldn22	0	0.04719212	0.079883969	0.009105591	-

										0.067573859
chr4	1.85E+08	1.85E+08	511	0.005	Stox2	31	0.010583934	0.096433022	0.081507317	0.046430409
chr4	1.85E+08	1.85E+08	374	0.006	Irf2	112511	0.044240008	0.068515138	0.017466763	0.083560547
chr4	1.86E+08	1.86E+08	143	0.005	Acs11	4433	0.21397322	0.051362717	0.241807049	0.054203499
chr4	1.86E+08	1.86E+08	1136	0.006	Acs11	1879	0.207983162	0.070745646	0.606290022	0.027547768
chr4	1.86E+08	1.86E+08	766	0.035	Acs11	55713	0.174978393	0.035307295	0.122741315	0.034136668

[0165] Table 8. Enrichment of cross-species DMRs over DIAGRAM GWAS loci, related to Figure 3.

DIAGRAM		Number of GWAS		Number of GWAS	
Cutoff	# Loci	Adipose	Islet	Adipose	Islet
p<5e-08	18	2 (0.017)	0 (0.292)	1 (0.0113)	0 (0.108)
p<1e-07	20	2 (0.025)	0 (0.329)	1 (0.0154)	0 (0.121)
p<1e-06	35	3 (0.016)	1 (0.119)	1 (0.0376)	0 (0.179)
p<1e-05	215	6 (0.005)	2 (0.119)	2 (0.0253)	1 (0.058)
p<1e-04	428	9 (0.209)	5 (0.239)	3 (0.192)	2 (0.166)
p<1e-03	1601	24 (0.48)	12 (0.600)	6 (0.597)	7 (0.086)
p<1e-02	8353	77 (0.336)	39 (0.560)	27 (0.119)	12 (0.573)

[0166] This table summarizes the number and significance of overlaps of cross-species conserved adipose and pancreatic islet loci with DIAGRAM GWAS LD-blocks associated with SNPs at varying levels of significance as indicated by the cutoff column.

[0167] Table 9. Cross-species, directionally consistent DMRs that overlap with DIAGRAM T2D GWAS loci, related to Table 2.

Mouse DMR Chr	Mouse DMR start	Mouse DMR end	Mouse DMR width	Mouse DMR value	pval	qval	Nearest Gene	Nearest Gene Dist	Human Pval (for T2D)	DIAGRAM p-value of nearest GWAS loci	DIAGRAM SNP
chr3	97536603	97536878	276	0.093611	0.001563	0.012831	Arl6	34177	0.01811	0.00069	rs17302349
chr1	1.47E+08	1.47E+08	1014	0.079418	6.51E-05	0.002028	Bcl9	0	0.018785	0.00042	rs7512513

chr2	1.7E+08	1.7E+08	721	0.080218	6.51E-05	0.002028	G6pc2	0	0.005552	0.0034	rs16856159
chr11	17405939	17406583	645	0.080153	0.001497	0.012831	Kcnj11	3320	0.011108	4.40E-06	rs5215
chr11	17406741	17407082	342	0.077431	0.00293	0.017074	Kcnj11	2900	0.011108	4.40E-06	rs5215
chr11	17407373	17408268	896	0.081309	6.51E-05	0.002028	Kcnj11	1740	0.001281	4.40E-06	rs5215
chr11	27491277	27491838	562	-0.07805	0.000911	0.010693	Lgr4	2417	0.045056	0.0077	rs7945211
chr14	77511555	77511688	134	-0.08141	0.016081	0.045395	G18Rik	15082	0.024935	0.0029	rs17752640
chr12	39297915	39298205	291	-0.07128	0.004232	0.020119	Cpne8	926	0.008167	1.60E-05	rs10506132
chr5	1.23E+08	1.23E+08	369	0.073114	0.001953	0.013892	Csnk1g3	263645	0.010391	0.00051	rs7708937

[0168] Similar to Table 3, this table lists pancreatic islet DMRs that are significant across species, directionally consistent, and overlap with DIAGRAM T2D LD blocks associated with nominally significant SNPs.

[0169] Table 10. Overlapping methylation change and adipose enhancer regions, related to Table 2.

[0170] This table displays the 171 cross-species conserved and directionally consistent regions with differential methylation along with the nearest enhancer and super enhancer found in adipose tissue (see Methods).

Mouse_chr	Mouse_start	Mouse_end	Mouse_Qval	Mouse_ Nearest_ Gene	Human_ Obesity_ Pval	Human_ Surgery_ Pval	distToNearest AdiposeEnhancer	distToNearest AdiposeSuper Enhancer
chr1	19804495	19805076	0.005	Capzb	0.009585	0.080255	0	0
chr1	48351513	48351750	0.011	OTTMUSG 00000008561	0.000272	0.105293	-546242	3080210
chr1	64061530	64062519	0.006	Pgm2	0.023275	0.223954	0	872196
chr1	1.11E+08	1.11E+08	0.009	Kcna3	0.00083	0.026296	199363	505540
chr1	1.14E+08	1.14E+08	0.006	Ptpn22	8.28E-05	0.111419	-13238	-2664547
chr1	1.14E+08	1.14E+08	0.005	Ptpn22	8.28E-05	0.111419	-14457	-2665766
chr1	1.14E+08	1.14E+08	0.006	Ptpn22	8.28E-05	0.111419	-15365	-2666674
chr1	1.5E+08	1.5E+08	0.012	Plekho1	0.011516	0.092524	0	-140802
chr1	1.51E+08	1.51E+08	0.005	Tnfaip8l2	2.58E-05	0.043103	-10710	123288
chr1	1.61E+08	1.61E+08	0.005	Cd48	0.037991	0.058607	-131593	479879

chr1	1.61E+08	1.61E+08	0.005	Arhgap30	4.80E-09	0.010337	0	123016
chr1	1.61E+08	1.61E+08	0.006	Fcgr3	0.002653	0.017223	23356	-301163
chr1	1.93E+08	1.93E+08	0.006	Rgs1	0.015727	0.016068	-14758	-8658971
chr1	1.99E+08	1.99E+08	0.005	Ptpnc	0.018214	0.001093	16327	2807046
chr1	2.12E+08	2.12E+08	0.012	Nek2	0.012206	0.061637	-55976	531270
chr2	16084776	16085714	0.024	Mycn	0.026463	0.020034	-238915	703959
chr2	47216781	47216960	0.024	Ttc7	0.00172	0.177315	0	0
chr2	62426656	62427018	0.012	B3gnt2	0.021727	0.007112	-588	494136
chr2	67625367	67625994	0.011	Etaal	0.014192	0.057427	-136215	789174
chr2	68592241	68593052	0.005	Plek	0.000418	0.001208	0	45321
chr2	68961351	68961704	0.012	Arhgap25	0.000287	0.009437	28829	-281076
chr2	68962521	68963097	0.005	Arhgap25	0.000287	0.009437	27436	-282246
chr2	1.01E+08	1.01E+08	0.03	Lonrf2	0.037745	0.308483	49822	1884353
chr2	1.45E+08	1.45E+08	0.011	Zeb2	0.013433	0.433484	0	-129373
chr2	1.58E+08	1.58E+08	0.005	Pscdbp	0.003846	0.009592	53777	-969584
chr2	1.58E+08	1.58E+08	0.005	Pscdbp	0.003846	0.009592	52287	-971607
chr2	1.77E+08	1.77E+08	0.033	Hoxd3	0.007824	0.00978	-17061	-2094046
chr6	1608907	1609124	0.033	Foxc1	0.021499	0.02655	2698	1409463
chr6	26595986	26596063	0.044	Abt1	0.001774	0.029453	-22946	-1872832
chr6	75913118	75913414	0.011	Col12a1	0.039965	0.018634	0	12455722
chr6	79922240	79922533	0.009	Hmgn3	0.031224	0.133123	19305	8446603
chr6	1.08E+08	1.08E+08	0.011	Scml4	0.000254	0.001184	128176	732446
chr6	1.64E+08	1.64E+08	0.005	Qk	0.005485	0.039999	0	0
chr8	1715132	1715419	0.012	Cln8	0.020312	0.19778	0	5531120
chr8	41907137	41907600	0.006	Myst3	0.033952	0.032882	0	-3240680
chr8	66701734	66702089	0.006	Pde7a	0.000239	0.022706	50510	-4064608
chr8	70588403	70588535	0.047	Slco5a1	0.037372	0.027051	-70101	6994966
chr8	79577640	79577971	0.012	3110050N22Rik	0.027335	0.01307	-49109	-1965288
chr8	1.29E+08	1.29E+08	0.026	Myc	0.029892	0.01329	-1615	2002110
chr8	1.31E+08	1.31E+08	0.03	0910001A06Rik	0.006433	0.053705	0	0
chr8	1.45E+08	1.45E+08	0.012	AA409316	0.02697	0.004606	-100509	-1E+07
chr10	6190543	6191242	0.006	Rbm17	0.017146	0.019469	0	0

chr10	97144444	97145224	0.005	Sorbs1	0.027219	0.001956	0	0
chr10	97517128	97517433	0.032	Entpd1	0.026474	0.061996	-14493	-308852
chr10	98416374	98417030	0.006	Pik3ap1	0.002274	0.11671	15807	-1208098
chr10	1.05E+08	1.05E+08	0.005	As3mt	0.004186	0.018854	-53262	-53262
chr10	1.05E+08	1.05E+08	0.026	Sh3pxd2a	0.000644	0.000123	0	229078
chr10	1.15E+08	1.15E+08	0.035	Tcf7l2	0.000356	0.353841	0	0
chr10	1.15E+08	1.15E+08	0.009	Tcf7l2	0.001798	0.008486	0	0
chr10	1.3E+08	1.3E+08	0.005	Ptpre	0.006559	0.022389	0	1885307
chr10	1.34E+08	1.34E+08	0.046	Bnip3	0.032971	0.02267	0	-2028713
chr10	1.35E+08	1.35E+08	0.006	6430531B16Rik	0.047166	0.027803	0	-3307728
chr12	1663136	1663829	0.005	Wnt5b	0.043716	0.049488	21029	102052
chr12	7060921	7061200	0.032	Ptpn6	0.000294	0.013488	501	-578219
chr12	8275965	8276317	0.026	Clec4a4	0.013804	0.010436	32585	-1793263
chr12	15111717	15112453	0.005	Arhgdib	0.009564	0.029894	0	1386708
chr12	26392741	26393036	0.031	Sspn	0.032866	0.136648	0	-111890
chr12	62658334	62658491	0.047	Usp15	0.01282	0.018703	272793	-2525517
chr12	69140644	69140871	0.022	Slc35e3	0.002949	0.174568	-62479	119374
chr12	75874872	75875171	0.005	Glipr1	0.028933	0.133593	0	-3198773
chr12	95000081	95001986	0.026	Tmcc3	0.004387	0.167216	6592	-1200387
chr12	1.03E+08	1.03E+08	0.005	Pmch	0.00435	0.264568	-49193	173265
chr12	1.04E+08	1.04E+08	0.017	Tdg	0.005058	0.05672	-1480	-1484292
chr12	1.15E+08	1.15E+08	0.033	Tbx3	0.039599	0.080103	0	-2877534
chr12	1.15E+08	1.15E+08	0.017	Tbx3	0.002757	0.001537	-2373	-2894447
chr12	1.15E+08	1.15E+08	0.005	Tbx3	0.000306	0.001286	-5650	-2897724
chr12	1.19E+08	1.19E+08	0.012	Taok3	0.030025	0.024758	0	-293657
chr22	40858582	40860665	0.005	Mkl1	0.001385	0.019491	-17872	-3262460
chr17	1220102	1220359	0.005	Tusc5	0.004981	0.331278	0	0
chr17	7483300	7483645	0.029	Cd68	0.004383	0.004552	0	252958
chr17	8027102	8027420	0.029	Hes7	0.039742	0.158273	2176	-277411
chr17	34415897	34416118	0.026	Ccl3	0.001156	0.04389	-114527	1419299
chr17	34417557	34418034	0.009	Ccl3	0.001156	0.04389	-116187	1417383
chr17	47819090	47819795	0.005	5730593F17Rik	0.0074	0.007408	0	-1152018

chr17	56355449	56355594	0.044	Mpo	0.038596	0.084256	57556	1050065
chr17	79419543	79420749	0.006	Bahcc1	0.017499	0.024719	13038	-15458
chr17	80052124	80053059	0.009	Fasn	0.002345	0.045378	862	-648039
chr5	34655547	34655935	0.029	Rai14	0.044333	0.057769	301318	-2479732
chr5	34687673	34687965	0.029	Rai14	0.027203	0.000317	269288	-2511858
chr5	66491448	66492361	0.005	Cd180	0.010515	0.032888	-981	-161032
chr5	67585536	67585875	0.033	Pik3r1	0.027604	0.050059	0	0
chr5	77803248	77803367	0.03	Lhfpl2	0.014339	0.000121	0	0
chr5	1.07E+08	1.07E+08	0.011	Efna5	0.04026	0.005299	0	8368099
chr5	1.08E+08	1.08E+08	0.011	Fert2	0.01459	0.032578	0	7190713
chr5	1.48E+08	1.48E+08	0.006	Adrb2	0.005235	0.055308	0	304318
chr5	1.49E+08	1.49E+08	0.006	Csf1r	0.0083	0.011174	25190	398535
chr5	1.7E+08	1.7E+08	0.011	Kcnmb1	1.42E-05	0.006693	-564	2397729
chr5	1.79E+08	1.79E+08	0.012	Adams2	0.010601	0.004506	-49139	-49139
chr7	1408433	1408951	0.006	Micall2	0.010304	0.002223	89119	5005012
chr7	26460385	26460633	0.015	Snx10	0.017227	0.02051	-20840	-217203
chr7	27201206	27201499	0.027	Hoxa9	0.000244	0.000896	0	0
chr7	27202553	27202993	0.044	Hoxa9	0.007726	0.098969	0	0
chr7	27203073	27203466	0.008	Hoxa9	0.007726	0.098969	0	0
chr7	50345715	50346835	0.005	Ikzf1	0.000204	0.000649	-235589	-2784315
chr7	73624879	73625346	0.005	Lat2	0.025835	0.073432	-14413	415807
chr7	76828348	76828576	0.042	Fgl2	0.032838	0.072158	16610	-570377
chr7	1.07E+08	1.07E+08	0.005	Prkar2b	0.001245	0.052519	0	0
chr7	1.4E+08	1.4E+08	0.005	Tbxas1	7.05E-05	0.012069	-51336	-74443
chr14	75361059	75361571	0.033	Dlst	0.002412	0.006712	-1825	-1098230
chr14	77791405	77791671	0.011	Gstz1	0.010969	0.049885	-8940	-265105
chr14	89707672	89708616	0.009	Foxn3	0.005944	0.097831	-13657	1138563
chr14	93117257	93117768	0.029	Rin3	0.003787	0.012258	-63158	411618
chr14	1.01E+08	1.01E+08	0.006	Evl	0.003391	0.00473	9386	2104521
chr9	14203697	14204081	0.005	Nfib	0.001304	0.009562	0	0
chr9	32457089	32457364	0.005	Ddx58	0.019333	0.303952	-76	-76
chr9	95726604	95726801	0.03	Fgd3	0.000783	0.106858	-25224	-1533887

chr9	1.14E+08	1.14E+08	0.006	Gng10	0.008616	0.004046	0	-1506896
chr9	1.16E+08	1.16E+08	0.033	Rgs3	0.034041	0.07686	0	0
chr9	1.18E+08	1.18E+08	0.016	Tnfsf8	0.000934	0.010527	0	-531146
chr9	1.31E+08	1.31E+08	0.005	Spna2	0.021423	0.017082	0	-503909
chr13	36049213	36050887	0.005	Mab21l1	0.000513	0.000333	0	-2183260
chr13	40943529	40943850	0.006	Foxo1	0.045248	0.09776	0	175911
chr13	46751279	46752608	0.005	Lcp1	0.014544	0.021646	-5166	2041932
chr13	46753288	46754147	0.009	Lcp1	0.014544	0.021646	-7175	2040393
chr13	50204794	50205145	0.022	Arl11	0.04428	0.046926	-4181	-1375720
chr3	9911613	9911971	0.029	Cidec	0.014016	0.028881	0	0
chr3	24869934	24870287	0.008	Rarb	0.029778	0.111071	-186244	4450134
chr3	1.13E+08	1.13E+08	0.005	Cd200rl	0.009567	0.011117	42134	-318068
chr3	1.13E+08	1.13E+08	0.008	Boc	0.026238	0.048245	339	488239
chr3	1.5E+08	1.5E+08	0.012	2810407C02Rik	0.000504	0.000438	0	-184142
chr3	1.57E+08	1.57E+08	0.006	Veph1	0.000152	0.004779	-33016	-430940
chr16	16123803	16124667	0.005	Abcc1	0.028138	0.092617	0	-1689502
chr16	50715116	50715299	0.011	9130017C17Rik	0.000205	0.012381	13526	-2286067
chr16	55540360	55540833	0.006	Mmp2	0.012314	0.083101	-17985	-2364486
chr16	56137894	56138256	0.006	Gnao1	0.004769	0.003215	-266909	-2962020
chr16	69457347	69457490	0.044	Cyb5b	0.012444	0.184651	-5180	-1121558
chr16	87903345	87904117	0.006	BC048644	0.034324	0.034225	47037	-2293210
chr16	88770501	88770848	0.012	Rnfl66	0.04324	0.137684	0	-3160366
chr21	36398548	36398828	0.011	Runx1	0.000152	0.001644	20276	-134293
chr21	36420456	36421805	0.005	Runx1	4.31E-06	0.00056	0	-156201
chr21	39868683	39869555	0.01	Erg	0.000108	0.004244	0	0
chr18	56809110	56809370	0.017	Sec11c	0.047576	0.081715	-86050	-3789034
chr19	15574480	15574831	0.046	A430107D22Rik	0.019917	0.084596	-10634	-153351
chr19	40750567	40750963	0.015	Akt2	0.005362	0.026742	0	0
chr19	49841249	49841895	0.009	Cd37	0.027967	0.000388	493	-1591657
chr19	51645546	51714605	0.006	Cd33	0.026485	0.042378	-3881	-3395954
chr19	54876429	54876765	0.03	Lair1	0.02649	0.000733	0	-6626837
chr11	6267881	6268720	0.012	Cnga4	0.001427	0.095175	-19648	354360

chr11	10316893	10317243	0.035	Adm	0.015286	0.074089	0	0
chr11	10324978	10325218	0.033	Adm	0.006833	0.022077	0	0
chr11	47415025	47415403	0.006	Sfpil	0.005613	0.020093	0	100118
chr11	63528336	63528808	0.006	2700081O15Rik	0.009375	0.019784	5253	-36600
chr11	66673568	66673798	0.043	Pcx	0.049345	0.126683	0	0
chr11	67036383	67036711	0.029	Adrbk1	0.000862	0.093207	0	-332714
chr11	67165584	67166226	0.009	Ppp1ca	0.038535	0.003821	-23002	-461915
chr11	73692113	73692198	0.028	Ucp2	0.048033	0.079853	-2531	1319884
chr11	1.18E+08	1.18E+08	0.022	Il10ra	0.000608	0.020984	0	613105
chr11	1.23E+08	1.23E+08	0.015	Bsx	0.034711	0.01019	-44045	78806
chr15	37173455	37174113	0.026	Meis2	0.028601	0.011445	210804	2244812
chr15	61136559	61136807	0.024	Rora	0.0012	0.091815	-42316	-416528
chr15	96902600	96903113	0.005	Nr2f2	4.02E-05	0.001088	-1250	-1250
chr15	96905568	96906197	0.006	Nr2f2	0.000917	7.85E-06	-4218	-4218
chr15	96907528	96907914	0.022	Nr2f2	0.000917	7.85E-06	-6178	-6178
chr20	18269464	18269677	0.029	6330439K17Rik	0.004138	0.002225	31641	1674677
chr20	25033629	25034262	0.005	Acssl	0.035246	0.043383	0	-5041490
chr20	25251494	25252151	0.005	Pygb	0.009127	0.014408	831	5027244
chr20	39314354	39315324	0.006	Mafb	0.016336	0.014954	0	3884911
chr20	47803610	47804113	0.006	Stau1	0.048578	0.316221	0	485791
chr20	48924232	48924768	0.022	Cebpb	0.004243	0.078455	0	0
chr20	56135219	56135721	0.006	Pck1	0.008352	0.046531	0	122179
chr20	56135935	56137320	0.005	Pck1	0.003188	0.040125	0	120580
chr20	56138648	56138937	0.016	Pck1	0.003188	0.040125	0	118963
chr20	56139464	56139982	0.009	Pck1	0.003188	0.040125	0	117918
chr4	38858508	38859749	0.008	Tlr6	0.003242	0.050924	0	-157838
chr4	84034389	84034401	0.015	Plac8	0.000416	0.009649	-99147	3885033
chr4	1.09E+08	1.09E+08	0.024	Hadh	0.044534	0.171435	0	0
chr4	1.14E+08	1.14E+08	0.005	Larp7	0.027309	0.053868	-15696	491614
chr4	1.84E+08	1.84E+08	0.006	Cldn22	0.047192	0.009106	75490	1424121
chr4	1.85E+08	1.85E+08	0.005	Stox2	0.010584	0.081507	-21381	890761
chr4	1.85E+08	1.85E+08	0.006	Irf2	0.04424	0.017467	0	361837

[0171] Table 11. Human Subject Information, related to Experimental Procedures.

[0172] This table displays relevant information about the human subjects examined in this study.

Patient Number	Obesity	Type 2 Diabetes	Age at surgery	BMI	Waist circumference (cm)	pre-op HDL (mol/L)	pre-op LDL (mol/L)	pre-op Cholesterol (mol/L)	Preop HbA1C mmol/mol	pre-op HbA1c (%)
All Male										
1	Ob	T2D	42	37.00	118	1	1.3	2.9	48	5.6
2	Ob	T2D	62	36.00	143	0.6	2	3.6	43	5.2
3	Ob	ND	43	40.30	137	2	3.4	5.8	39	4.8
4	Ob	T2D	56	37.10	124	0.9		4	71	7.8
5	Ob	ND	36	41.60	125	1.1	3.7	5.5	34	4.3
6	Ob	T2D	52	42.90	140	0.9	3.7	5.2	52	6
7	Ob	ND	49	40.00	140	1.4	3.8	6.1	40	4.9
8	Ob	T2D	41	40.40	125	3.9	3.7	5	69	7.6
9	Ob	ND	50	35.60	122	0.4	2.3	3.9	41	5
10	Ob	T2D	34	40.30	139	1	4.9	6.9	48	5.6
11	Ob	T2D	37	42.70	139	0.7	1.7	3.6	49	5.7
12	Ob	T2D	36	45.84	143	0.6	3.6	4.9		5.6
13	Ob	ND	41	37.22	122	1.1	4.4	6.2	4.4	5.8
14	Ob	ND	59	37.24	120	1	3.1	4.9		4.2
15	L	ND	39	24.50	87	1.1	3.9	6	38	4.7
16	L	ND	41	27.80	89	0.9	3.1	4.6	37	4.6
17	L	ND	59	25.10	100	1.3	4.7	6.5	36	4.5
18	L	ND	36	26.30	99	1.4	3.6	5.3	41	5
19	L	ND	39	28.40	102	1	3	4.6	37	4.6
20	L	ND	49	22.60	80	1.5	3.7	5.7	38	4.7
21	L	ND	41	20.00						

[0173] Table 12. Human Subject Information, related to Experimental Procedures.

[0174] This table displays relevant information about the human subjects examined in this study.

Patient Number	pre-op glucose (mmol/L)	insulin pre-op (pg/ml)	insulin pre-op (pmol/L)	HOMA-IR pre-op	preop TG (mol/L)	insulin 2 days after RYGB (pmol/L)	HOMA-IR 2 days after RYGB	follow up time (month)	Weight follow-up (kg)	BMI follow-up	Waist follow up (cm)
All Male											
1	6.7				1.2						
2	6.7	1139.035	196.1148	45.32432	3.3	118.6123	1033.85	8	128.1	31.4	129
3	5.5	721.27	124.1856	26.49293	0.9	140.6043	776.046	7	112.5	31.5	
4	17.3	893.445	153.8301	53.32775	6.1	114.697	784.1708	7	87.9	28.4	100
5	5.4	1068.2	183.9187	35.14891	1.6						
6	7.5	973.01	167.5293	44.67447	1.2	182.0532	1355.522	6	115.2	34.8	119
7	5.9	579.32	99.74518	21.72228	2	100.1154	443.8233	5	112	32	112
8	8.2	850.8	146.4876	49.48026	1.4	131.0348	853.1098	7	98.8	31.9	118
9	5.3	893.095	153.7698	34.17107	2.6						
10	7.9	1066.505	183.6269	45.70269	2.2	227.0377	1852.899	9	96.2	30	108
11	8.2	1347.56	232.0179	58.77787	2.5	178.3385	1839.01	5	127.8	35.4	122
12	6.6	1740.36	299.6488	74.57925	1.5	177.1935	2359.814				
13											
14	5.2	1160.125	199.746	37.28593	1.9	241.5694	2144.557				
15	5.9	87.675	40	8.4	2.2						
16	5.7	561.845	189	38.6	1.3						
17	4.8	261.13	62.3	12.5	1						
18	5.3	375.42	64.6	14.4	0.92						
19	5.5	363.32	62.6	12.8	1.3						
20	5.9	240.46	41.4	8.6	1						
21			47.2								

[0175] Although the invention has been described with reference to the examples herein, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

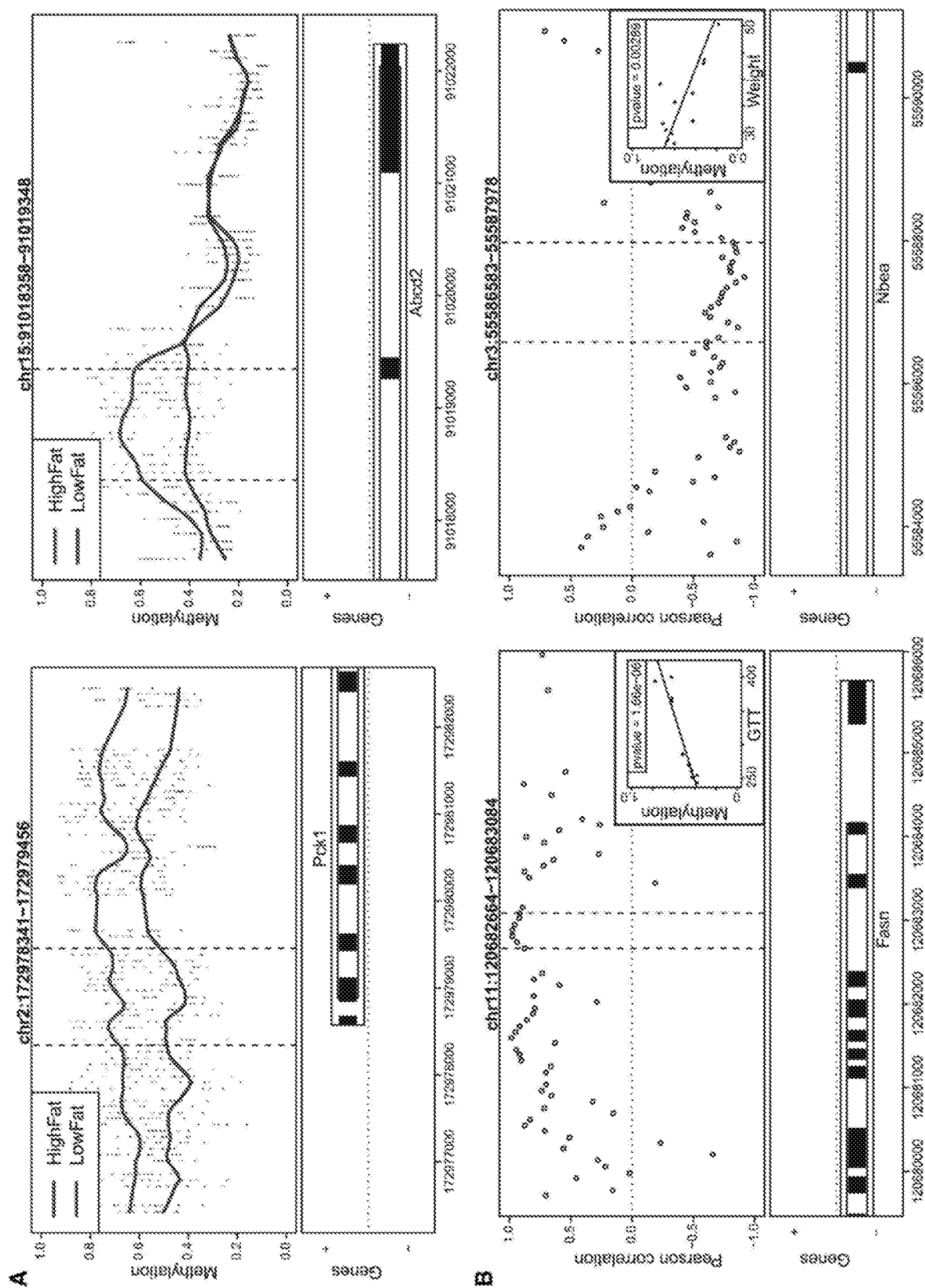
What is claimed is:

1. A method for identifying a subject having or at risk of having a metabolic disease comprising identifying in the subject one or more genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease, thereby identifying the subject as having or at risk of having a metabolic disease.
2. The method of claim 1, wherein the disease is diabetes or obesity.
3. The method of claim 2, wherein the disease is diabetes.
4. The method of claim 3, wherein the disease is type 2 diabetes (T2D).
5. The method of claim 1, wherein the genetic markers are hypermethylated or hypomethylated.
6. The method of claim 1, wherein the genetic markers are selected from 2 or more genes as set forth in Table 2.
7. The method of claim 4, wherein the genetic markers include at least Tcf7l2.
8. The method of claim 4, wherein the genetic markers are selected from Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prc1, Foxo1, Plekho1, Fasn, App, Akt2, or any combination thereof.
9. The method of claim 8, wherein the genetic markers are Mkl1, Plekho1 and Tnfaip8l2.
10. The method of claim 9, wherein the genetic markers are hypomethylated.
11. The method of claim 1, further comprising analyzing adipose cells of the subject, wherein an inflammatory response is a factor associated with having or risk of having T2D.
12. The method of claim 1, wherein identifying comprises determining methylation status of genetic markers.
13. The method of claim 12, wherein the methylation status is performed by one or more techniques selected from the group consisting of a nucleic acid amplification, polymerase chain reaction (PCR), methylation specific PCR, bisulfite pyrosequencing, single-strand conformation polymorphism (SSCP) analysis, restriction analysis, microarray technology, and proteomics.
14. The method of claim 1, wherein the genetic markers are identified from a sample from the subject, wherein the sample is selected from blood, adipose tissue, pancreatic tissue, liver tissue, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.

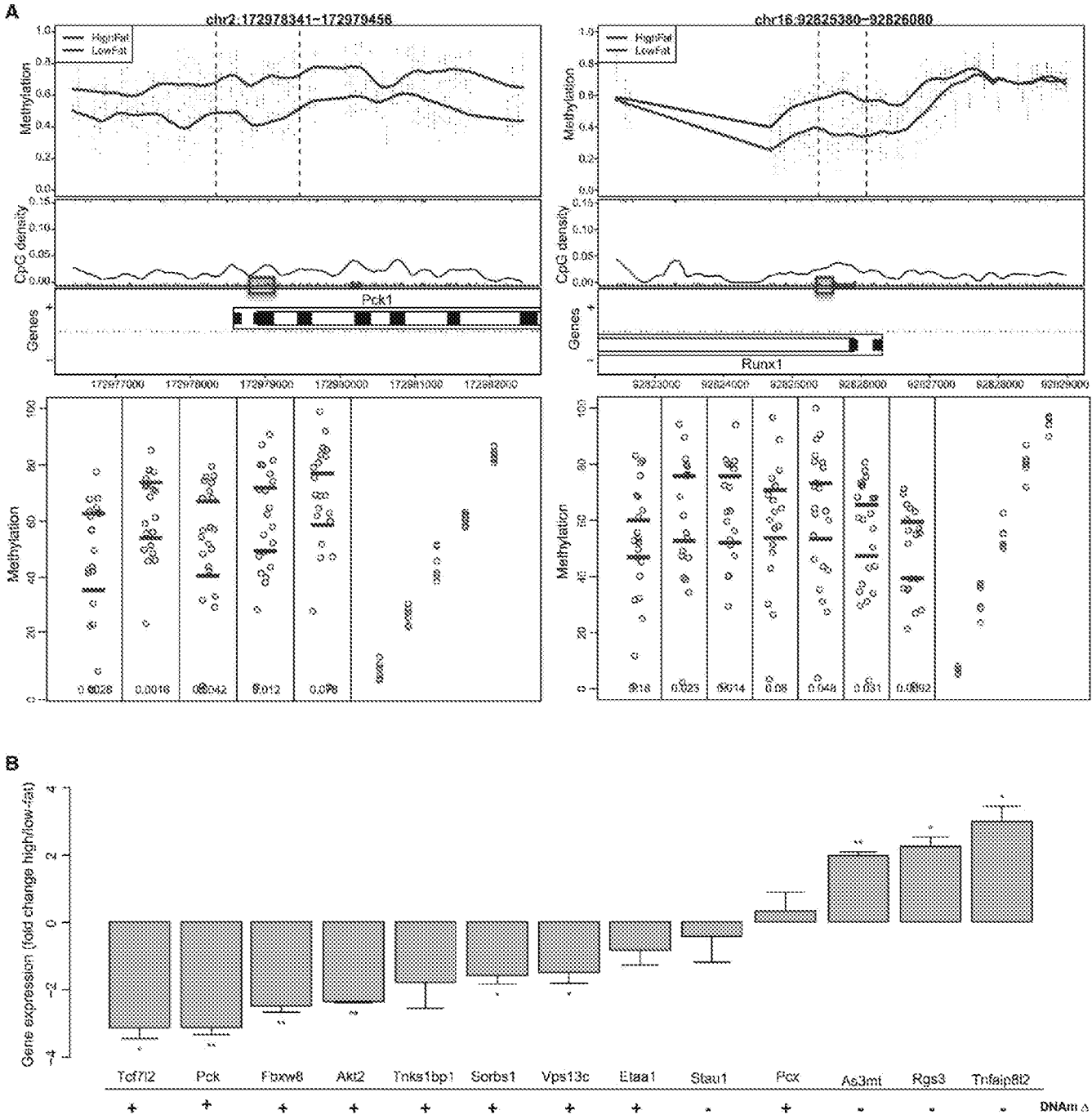
15. A method of treating a subject having or at risk of having a metabolic disease comprising increasing or decreasing gene expression of one or more genetic markers correlated with genetic risk loci for the subject based on an observation of hypomethylation or hypermethylation, respectively, of the marker, thereby treating the subject.
16. The method of claim 15, wherein the genetic markers affect glucose utilization by a cell.
17. The method of claim 15, wherein the genetic markers are associated with obesity.
18. The method of claim 15, wherein the genetic markers are associated with diabetes.
19. The method of claim 18, wherein the diabetes is type 2 diabetes (T2D).
20. The method of claim 15, wherein the genetic markers are selected from 2 or more genes as set forth in Table 2.
21. The method of claim 15, wherein the genetic markers include at least Tcf7l2.
22. The method of claim 15, wherein the genetic marker are selected from Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prc1, Foxo1, Plekho1, Fasn, App, Akt2, or any combination thereof.
23. The method of claim 22, wherein the genetic markers are Mkl1, Plekho1 and Tnfaip8l2.
24. The method of claim 23, wherein the genetic markers are hypomethylated.
25. The method of claim 15, wherein the genetic markers are identified from a sample from the subject, wherein the sample is selected from blood, adipose tissue, pancreatic tissue, liver tissue, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
26. A method of providing a prognostic evaluation of a subject having or at risk of having a metabolic disease comprising analyzing one or more genetic markers of the subject which is correlated with genetic risk loci prior to dietary and/or pharmaceutical intervention and following dietary and/or pharmaceutical intervention, and correlating a change in the genetic markers with a prognostic evaluation of the subject, thereby providing a prognostic evaluation.
27. The method of claim 26, wherein a decrease in expression of a marker previously up-regulated is correlated with improvement in the metabolic disorder.
28. The method of claim 26, wherein an increase in expression of a marker previously down-regulated is correlated with improvement in the metabolic disorder.
29. The method of claim 26, wherein the disease is diabetes or obesity.
30. The method of claim 29, wherein the disease is diabetes.

31. The method of claim 30, wherein the disease is type 2 diabetes (T2D).
32. The method of claim 26, wherein the genetic markers are hypermethylated or hypomethylated.
33. The method of claim 26, wherein the genetic markers are selected from 2 or more genes as set forth in Table 2.
34. The method of claim 33, wherein the genetic markers include at least Tcf7l2.
35. The method of claim 33, wherein the genetic markers are selected from Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prc1, Foxo1, Plekho1, Fasn, App, Akt2, or any combination thereof.
36. The method of claim 35, wherein the genetic markers are Mkl1, Plekho1 and Tnfaip8l2.
37. The method of claim 36, wherein the genetic markers are hypomethylated.
38. The method of claim 26, wherein the genetic markers are identified from a sample from the subject, wherein the sample is selected from blood, adipose tissue, pancreatic tissue, liver tissue, serum, urine, saliva, cerebrospinal fluid, pleural fluid, ascites fluid, sputum, and stool.
39. A method for identifying a subject having or at risk of having a metabolic disease, cancer, immune system disorder, cardiovascular disease, gastrointestinal disease or pulmonary disease comprising identifying in the subject genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject not having the disease.
40. The method of claim 39, wherein the metabolic disease is diabetes or obesity.
41. The method of claim 20, wherein the metabolic disease is diabetes.
42. The method of claim 41, wherein the metabolic disease is type 2 diabetes (T2D).
43. The method of claim 39, wherein the genetic markers are hypermethylated or hypomethylated.
44. The method of claim 39, wherein the genetic markers are selected from 2 or more genes as set forth in Table 2.
45. The method of claim 44, wherein the genetic markers include at least Tcf7l2.
46. The method of claim 44, wherein the genetic markers are selected from Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prc1, Foxo1, Plekho1, Fasn, App, Akt2, or any combination thereof.

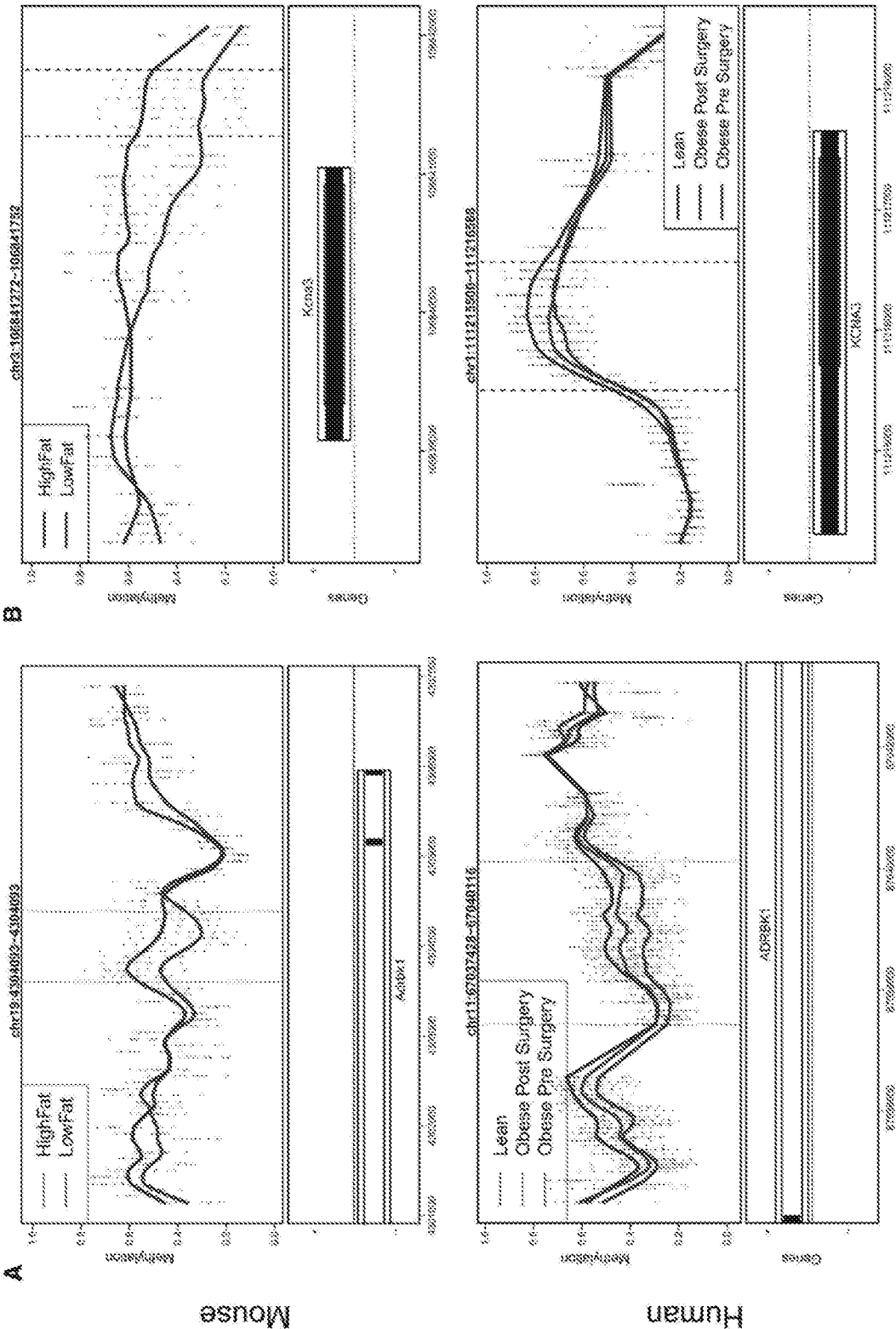
47. The method of claim 46, wherein the genetic markers are Mkl1, Plekho1 and Tnfaip8l2.
48. The method of claim 47, wherein the genetic markers are hypomethylated.
49. A method of determining a therapeutic regimen for a subject comprising identifying in the subject genetic markers correlating differentially methylated regions (DMRs) in the genome with genetic risk loci for the subject and comparing methylation patterns of the markers with a control sample from a subject thereby assessing the therapeutic regimen for the subject.
50. The method of claim 49, wherein the subject has, or is at risk of having a metabolic disease.
51. The method of claim 50, wherein the metabolic disease is diabetes or obesity.
52. The method of claim 51, wherein the metabolic disease is diabetes.
53. The method of claim 52, wherein the metabolic disease is type 2 diabetes (T2D).
54. The method of claim 49, wherein the genetic markers are hypermethylated or hypomethylated.
55. The method of claim 49, wherein the genetic markers are selected from 2 or more genes as set forth in Table 2.
56. The method of claim 55, wherein the genetic markers include at least Tcf7l2.
57. The method of claim 55, wherein the genetic markers are selected from Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prc1, Foxo1, Plekho1, Fasn, App, Akt2, or any combination thereof.
58. The method of claim 57, wherein the genetic markers are Mkl1, Plekho1 and Tnfaip8l2.
59. The method of claim 58, wherein the genetic markers are hypomethylated.



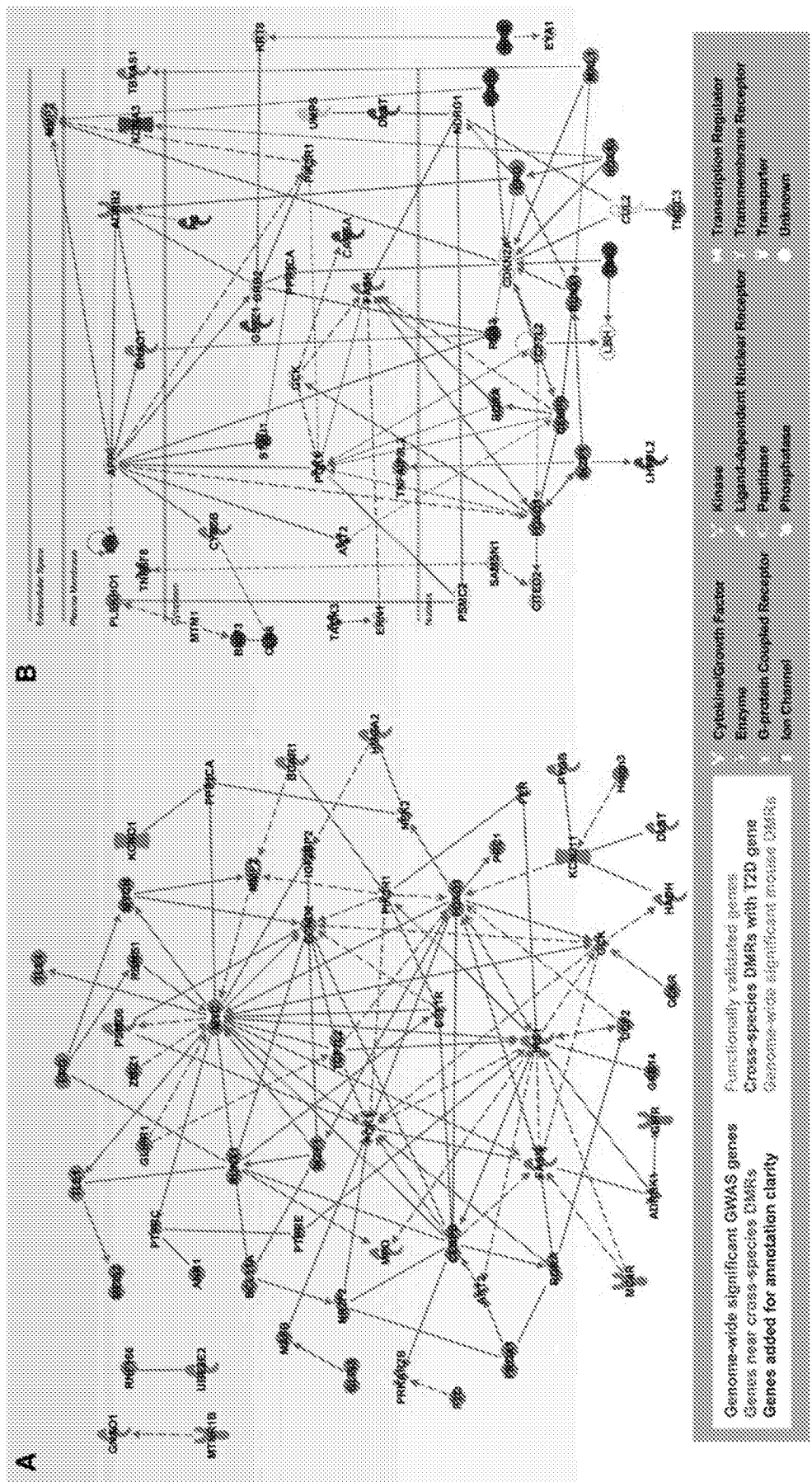
FIGS. 1A-1B



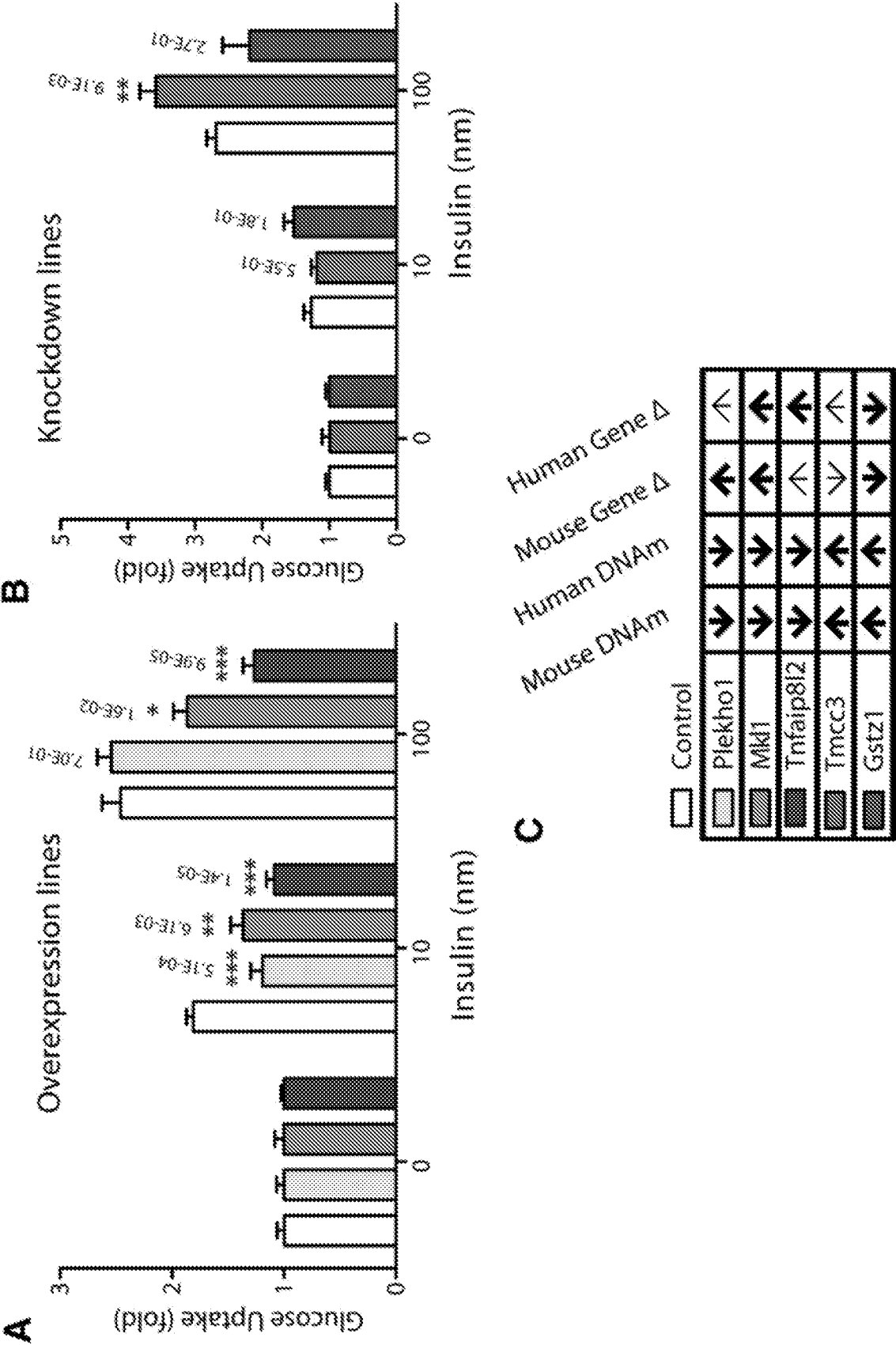
FIGS. 2A-2B



FIGS. 3A-3B



FIGS. 4A-4B



FIGS. 5A-5C

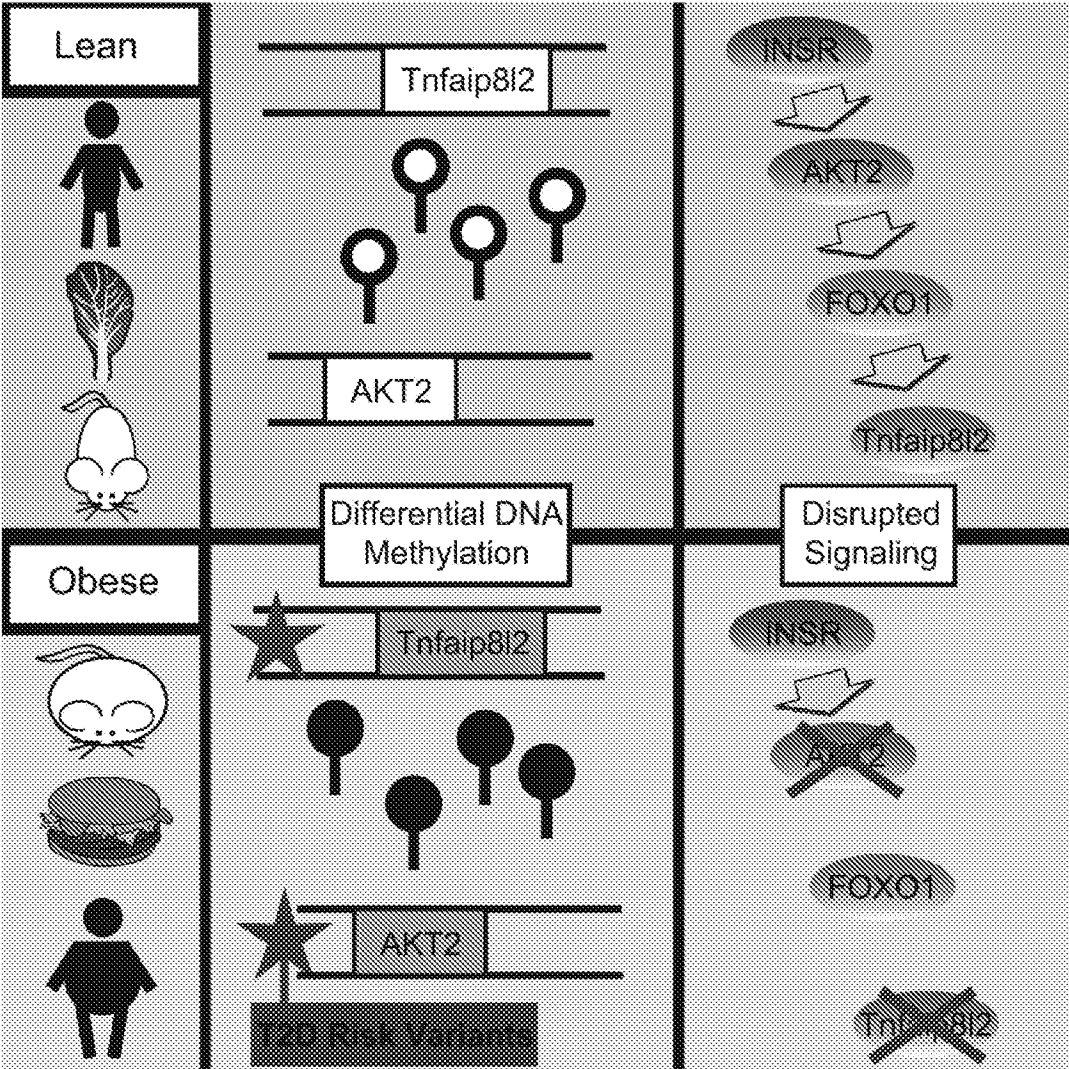


FIG. 6

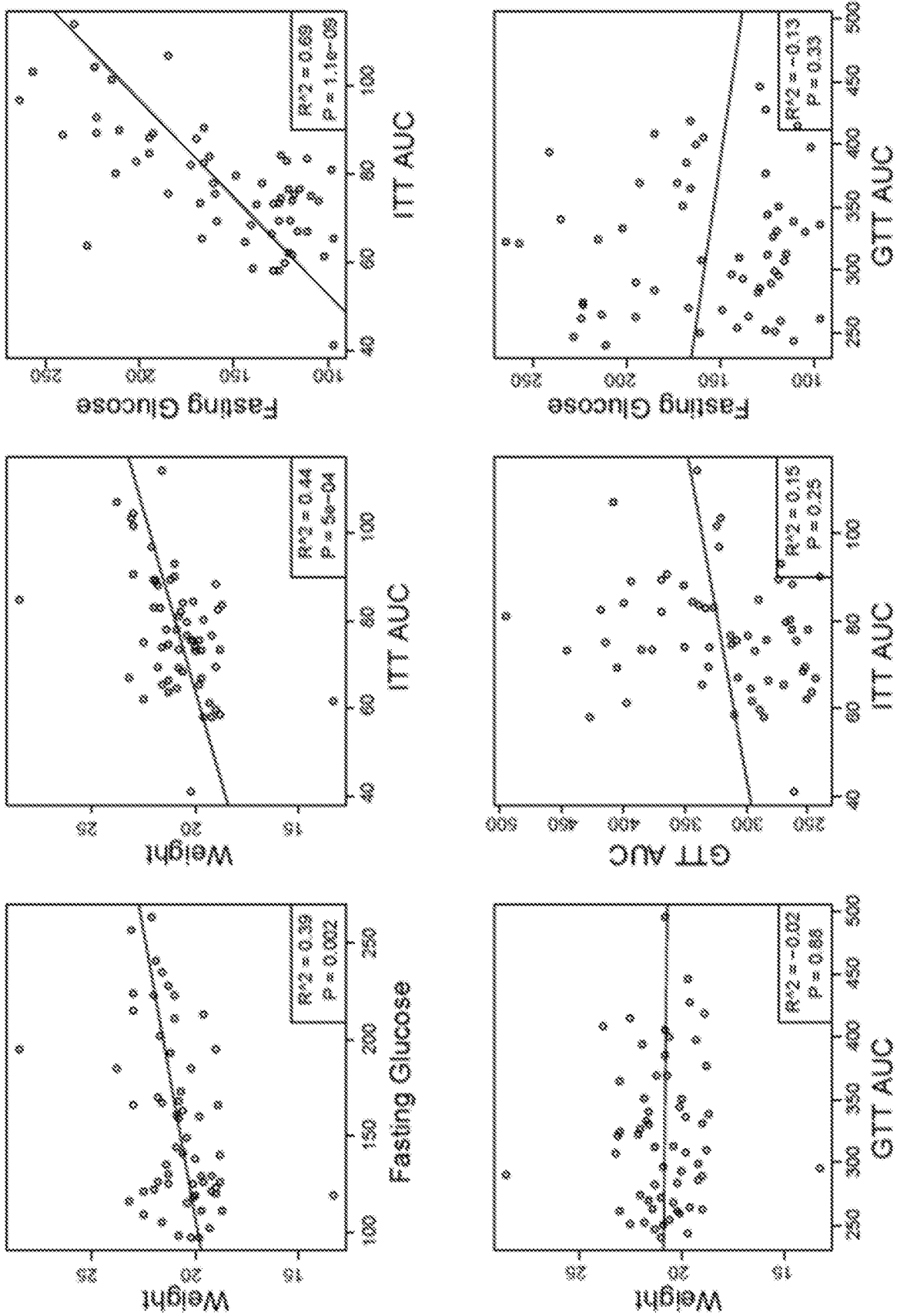
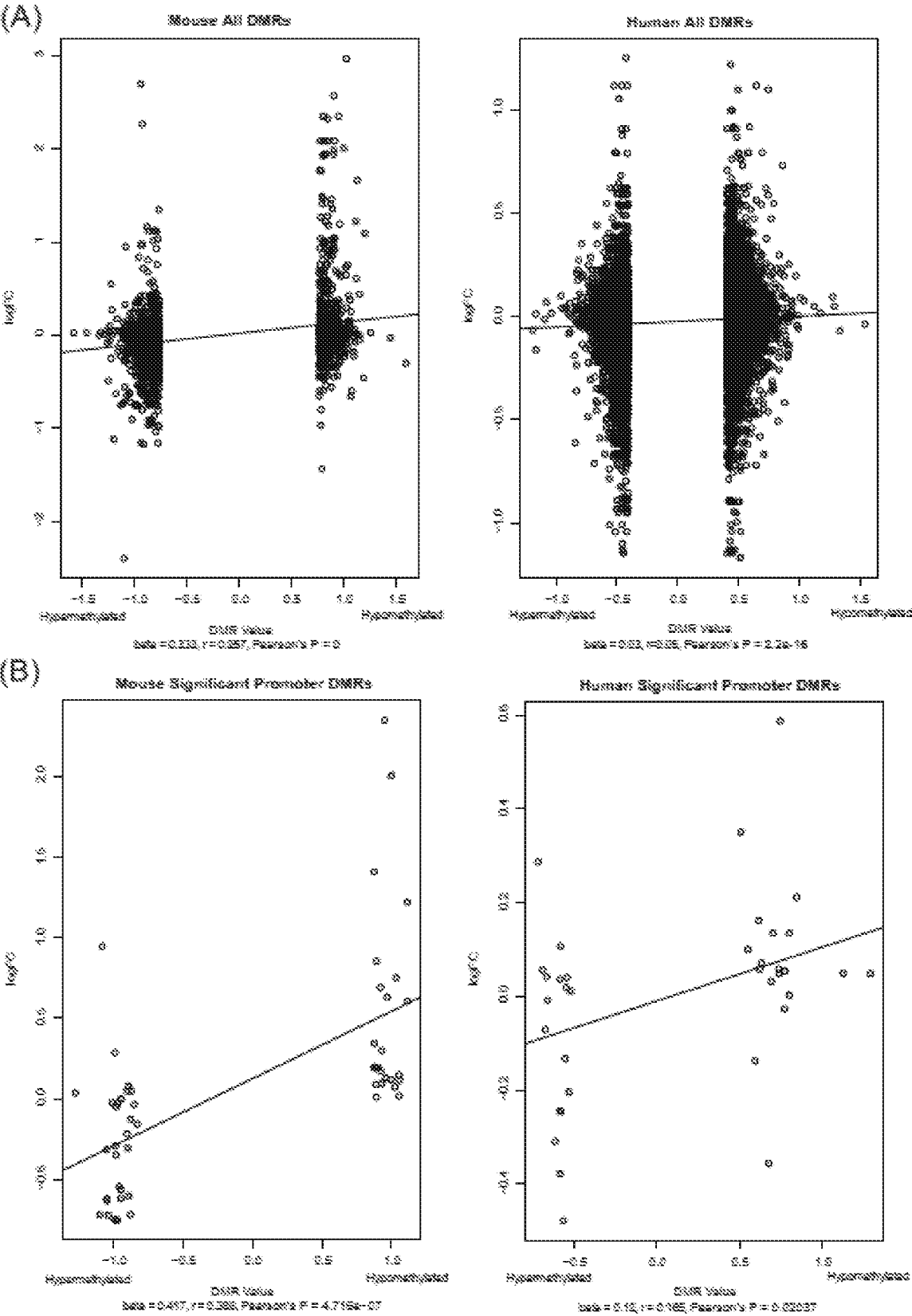
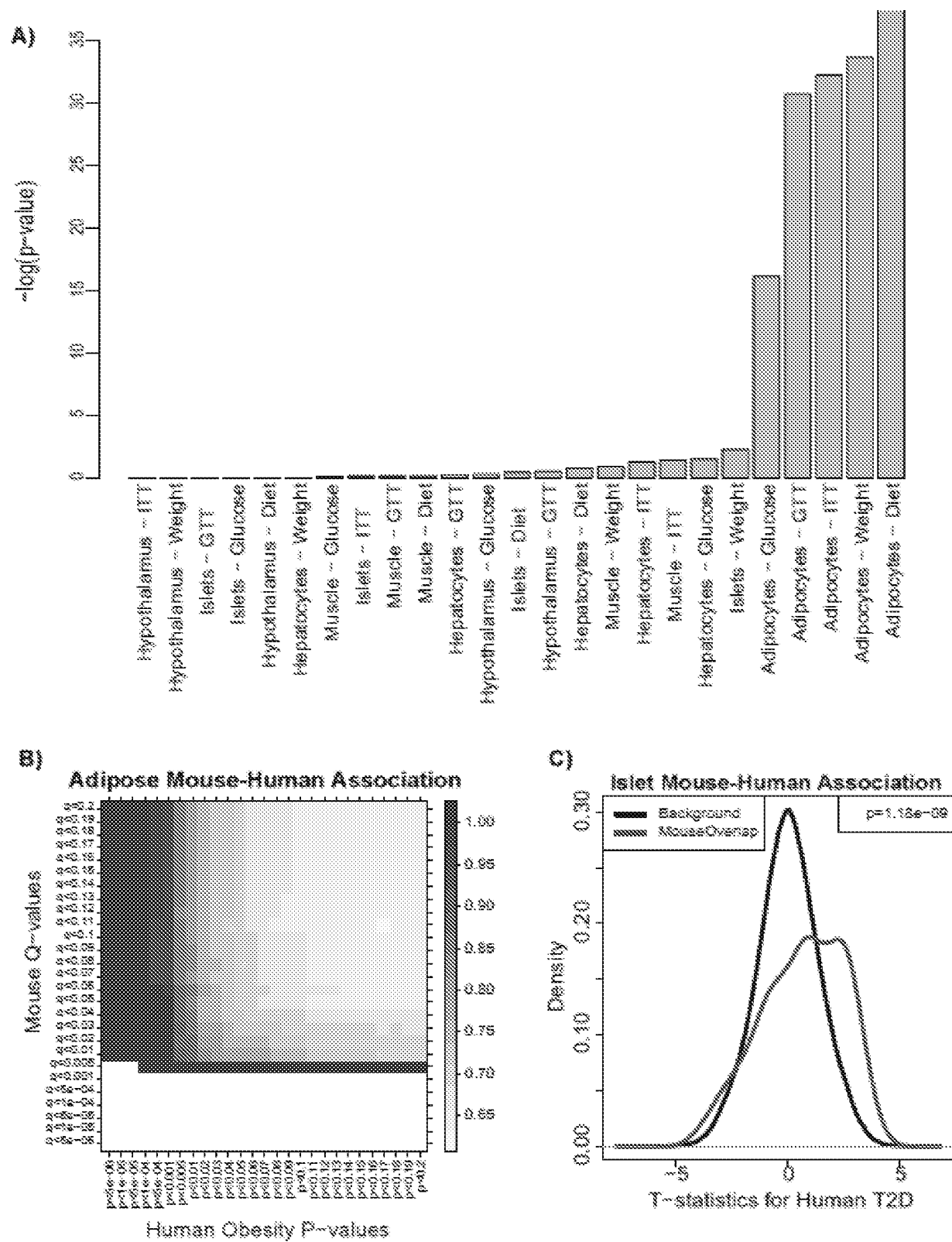


FIG. 7



FIGS. 8A-8B



FIGS. 9A-9C

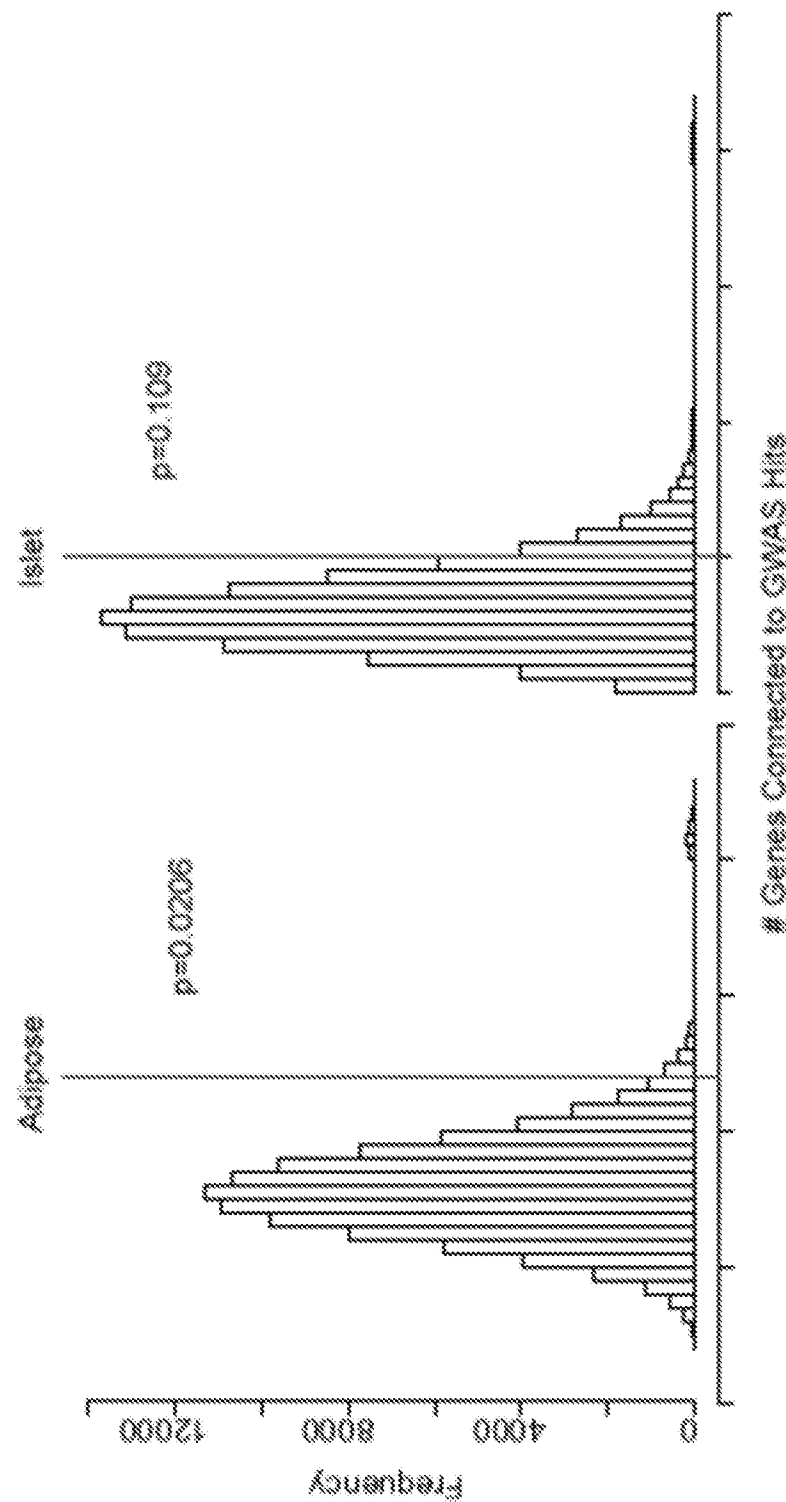


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/012217

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2016.01)

CPC - C12Q 1/6883 (2016.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07K 14/47, 16/18; C12N 15/09, 15/115, 5/071; C12Q 1/02, 1/04, 1/42, 1/68; G01N 27/62, 33/50, 33/53, 33/543 G01N 33 (2016.01)
CPC - C12Q 1/6883, 1/6886, 2600/112, 2600/158, 2600/16, 2600/178; G01N 2333/705, 33/5023, 33/53, 33/5308, 33/54326, 33/566, 33/574, 33/57419, 33/57434, 33/57484, 33/57488, 33/68, 33/6842, 33/6848, 33/6893 (2016.02)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC 435/6.1, 6.11, 6.12, 7.1, 7.23, 29; 436/501; 506/7, 9
IPC G01N 33/566, 33/57, 33/68, 37/00 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Google Scholar.

Search terms used: Mkl1, Plekho1, Tnfaip8l2, Tcf7l2, Prcl, Foxol, Fasn, App, Akt2 differentially methylated regions genome hypomethylated type 2 diabetes or obesity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2013/022995 A2 (CARIS LIFE SCIENCES LUXEMBOURG HOLDINGS, S.A.R.L. et al) 14 February 2013 (14.02.2013) entire document	1-3, 5, 6, 12-14, 26-30, 32-40, 43-52, 54-59
X -- Y	US 2012/0220477 A1 (STEINTHORSDDOTTIR et al) 30 August 2012 (30.08.2012) entire document	4, 7-11, 20, 22-24, 31, 41, 42, 53
Y	US 2013/0131140 A1 (NADLER et al) 23 May 2013 (23.05.2013) entire document	15-19, 21, 25
A	FAN et al. "Genome-Wide Screen of Promoter Methylation Identifies Novel Markers in Diet-Induced Obese Mice," Nutr. Hosp. 01 July 2014 (01.07.2014), Vol. 30, Pgs. 42-52. entire document	4, 7-10, 20, 22-24, 31, 41, 42, 53
A	LESCHE et al. "DNA Methylation Markers: a Versatile Diagnostic Tool for Routine Clinical Use". Current Opinion in Molecular Therapeutics," 01 June 2007 (01.06.2007), Vol. 9, Pgs. 222-30. entire document	11
		1-59
		1-59

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

11 April 2016

Date of mailing of the international search report

03 JUN 2016

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