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(71) Applicants: **THE BROAD INSTITUTE INC.** [US/US]; 415 Main Street, Cambridge, MA 02142 (US). **MAS-SACHUSETTS INSTITUTE OF TECHNOLOGY** [US/US]; 77 Massachusetts Ave., Cambridge, MA 02142 (US).

(72) Inventor: **HEIMAN, Myriam**; 33 Eliot Memorial Road, Newton, MA 02458 (US).

(74) Agents: **KOWALSKI, Thomas, J.** et al.; Vedder Price P.C., 1633 Broadway, New York, NY 10019 (US).

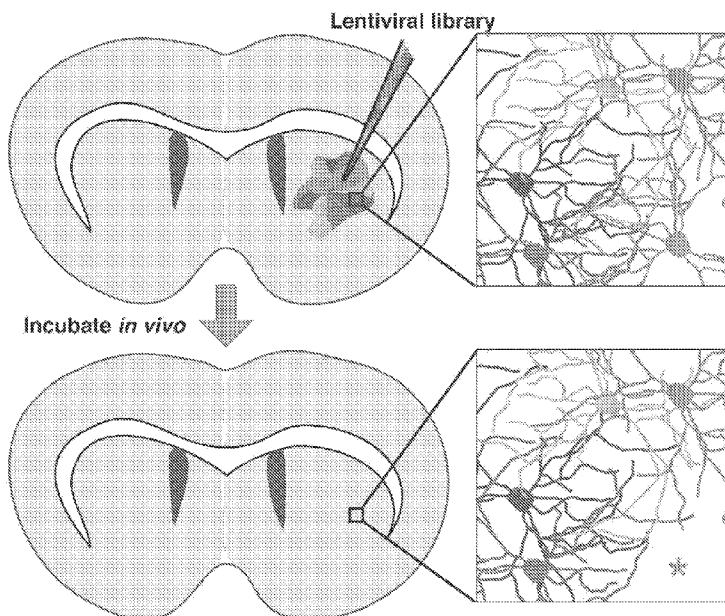
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[Continued on next page]

(54) Title: COMPOSITIONS, METHODS AND USE OF SYNTHETIC LETHAL SCREENING

Figure 2



(57) Abstract: The present invention generally relates to methods of identifying modulators of central nervous system diseases and the use of the modulators in treatment and diagnosis. The methods utilize a novel high throughput screen that includes injection of a library of barcoded viral vectors expressing shRNA's, CRISPR/Cas systems or cDNA's into animal models of disease and detecting synthetic lethality.

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**COMPOSITIONS, METHODS AND USE OF SYNTHETIC LETHAL SCREENING****RELATED APPLICATIONS AND INCORPORATION BY REFERENCE**

[0001] This application claims benefit of and priority to US provisional patent application Serial No. 62/122,686, filed October 27, 2014.

[0002] The foregoing applications, and all documents cited therein or during their prosecution (“appln cited documents”) and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein (“herein cited documents”), and all documents cited or referenced in herein cited documents, together with any manufacturer’s instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

**FEDERAL FUNDING LEGEND**

[0003] This invention was made with government support under grant number NS085880 awarded by the National Institutes of Health. The government has certain rights in the invention.

**FIELD OF THE INVENTION**

[0004] The present invention generally relates to methods of identifying modulators of central nervous system diseases using a novel high throughput methodology that includes expressing CRISPR/Cas systems, shRNA’s or cDNA’s in animal models of disease.

**BACKGROUND OF THE INVENTION**

[0005] Currently there are no cures or effective treatments for many neurodegenerative diseases. All of the major neurodegenerative diseases display characteristic nerve-cell (neuronal) vulnerability patterns, as well as an increased prevalence with advanced age. Many genes are involved in the pathogenesis of such diseases. As such, it is a challenge to find genes that are modulators of disease pathogenesis that can be used for diagnostic screening or effective treatments.

[0006] One such disease is Huntington’s Disease. Huntington’s disease, the most common inherited neurodegenerative disease, is characterized by a dramatic loss of deep-layer cortical

and striatal neurons, as well as morbidity in mid-life. Huntington's disease is the most common genetic cause of abnormal involuntary writhing movements called chorea.

[0007] Symptoms of the disease can vary between individuals and even among affected members of the same family, but usually progress predictably. The earliest symptoms are often subtle problems with mood or cognition. A general lack of coordination and an unsteady gait often follows. As the disease advances, uncoordinated, jerky body movements become more apparent, along with a decline in mental abilities and behavioral symptoms. Physical abilities are gradually impeded until coordinated movement becomes very difficult. Mental abilities generally decline into dementia. Complications such as pneumonia, heart disease, and physical injury from falls reduce life expectancy to around twenty years from the point at which symptoms begin. There is no cure for Huntington's disease, and full-time care is required in the later stages of the disease.

[0008] Treatments for Huntington's disease are available to reduce the severity of some of its symptoms (Frank et al., (2010) *Drugs* 70 (5): 561–71). Tetrabenazine was approved in 2008 for treatment of chorea in Huntington's disease in the United States. Other drugs that help to reduce chorea include neuroleptics and benzodiazepines. Compounds such as amantadine are still under investigation but have shown preliminary positive results (Walker, (2007) *Lancet* 369 (9557): 218-28). Hypokinesia and rigidity, especially in juvenile cases, can be treated with anti-Parkinson drugs, and myoclonic hyperkinesia can be treated with valproic acid.

[0009] Huntington's disease is caused by a mutation in the *Huntingtin* gene. Expansion of a CAG (cytosine-adenine-guanine) triplet repeat stretch within the *Huntingtin* gene results in a mutant form of the protein, which gradually damages cells in the brain, through mechanisms that are not fully understood. The length of the trinucleotide repeat accounts for 60% of the variation in the age symptoms appear and the rate they progress. The remaining variation is due to environmental factors and other genes that influence the mechanism of the disease (Walker, (2007) *Lancet* 369 (9557): 218-28).

[0010] The diagnosis of Huntington's disease is suspected clinically in the presence of symptoms. The diagnosis can be confirmed through molecular genetic testing which identifies the expansion in the *Huntingtin* gene. Testing of adults at risk for Huntington disease who have no symptoms (asymptomatic) of the disease has been available for over ten years. However, this testing cannot accurately predict the age a person found to carry a Huntington disease causing



mutation will begin experiencing symptoms, the severity or type of symptoms they will experience, or rate of disease progression. Other markers for disease progression are available, for example, loss of DARPP-32 striatal expression has been shown to be a molecular marker of Huntington's disease progression (Bibb et al., (2000) Proc Natl Acad Sci 6;97(12):6809-14).

[0011] Human genetic studies led to the identification of *huntingtin* as the causative gene. Recent genomic advances have also led to the identification of hundreds of potential interacting partners for huntingtin protein, and many hypotheses as to the molecular mechanisms whereby mutant huntingtin leads to cellular dysfunction and death (Goehler et al., (2004) Mol. Cell 15 (6): 853-65). Huntingtin protein is expressed in all mammalian cells and interacts with proteins which are involved in transcription, cell signaling and intracellular transporting (Harjes et al., (2003) Trends Biochem. Sci. 28 (8): 425-33). However, the multitude of possible interacting partners and cellular pathways affected by mutant huntingtin has obfuscated research seeking to understand the etiology of this disease, and to date no curative therapeutic exists for the disease.

[0012] A high throughput screening method to discover modulators of diseases, such as Huntington's disease, is a powerful tool to identify new drug targets, new prognostic methods, and new treatments.

[0013] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

#### SUMMARY OF THE INVENTION

[0014] It is an object of the invention to provide a genetic screening platform that could be used in mammals to identify modulators of diseases of the central nervous system. It is another object of the invention that the modulators are used in treatments, as therapeutic targets and for diagnosing disease.

[0015] In a first aspect, the present invention provides a method of screening for modulators of a disease comprising: administering to each of a first and second mammal of the same species at least one vector, each vector comprising a regulatory element operably linked to a nucleotide sequence that is transcribed in vivo, wherein the first mammal is a model of a human disease and the second mammal is a normal control mammal not a model of a human disease, and wherein the nucleotide sequence encodes a protein coding gene, or a short hairpin RNA, or a CRISPR/Cas system; harvesting DNA from the first mammal and the second mammal;

identifying the vectors by sequencing the harvested DNA; and comparing the representation of each vector from the first mammal and the second mammal, whereby a differential representation in the first mammal indicates that the protein coding gene, or short hairpin RNA target, or CRISPR/Cas system target is a modulator of the disease. Not being bound by a theory a synthetic lethal gene will be under represented in the first mammal that is a model of human disease. In a preferred embodiment, more than one vector is administered to each of a first and second mammal. In some embodiments, about 100, 500, 1000, 5000, 7000, 10,000, or 20,000 vectors may be administered to a mammal. The vectors may be administered stereotaxically. The nucleotide sequence that can be transcribed may target any gene within a genome or any sequence within a genome. The target sequence in the genome or target gene may be a regulatory sequence or any functional element in an RNA transcript or genomic locus, including, but not limited to a promoter, enhancer, repressor, polyadenylation signal, splice site, or untranslated regions. The gene may be any gene within a genome. The gene may be a peroxidase gene. The protein coding gene may be a cDNA, whereby a gene may be overexpressed. The vector may comprise a unique barcode sequence, and the method may further comprise identifying the barcodes during sequencing, whereby the identification of a barcode indicates the presence of a vector. A barcode can be any length nucleotide sequence within a polynucleotide that can be distinguished reliably by PCR, sequencing, or hybridization technology from similar length nucleotide sequences in another polynucleotide. The DNA sequencing may be any sequencing technique, preferably next generation sequencing, such as, Illumina sequencing. The barcodes may be identified by microarray analysis. Microarrays may be constructed such that cDNA complementary to the sequences of the barcodes are bound to the microarray. Harvested genomic DNA is hybridized to the bound cDNA to determine the amount of each barcode. Additionally, genomic DNA from the first mammal and second mammal are fluorescently labelled with different fluorescent dyes. For example one dye can fluoresce red and the other green. Both sets of labelled genomic DNA can then be hybridized to the same microarray and fluorescence can be compared to determine barcode representation.

[0016] The CRISPR/Cas system may comprise: a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence comprising at least one guide sequence, a tracr RNA, and a tracr mate sequence, wherein the at least one guide sequence hybridizes with a target sequence; and a second regulatory element operably linked to a

nucleotide sequence encoding a Type II Cas9 protein. The first and second mammals may be transgenic non-human mammals comprising Cas9 and the nucleotide sequence encoding a CRISPR/Cas system may comprise at least one guide sequence, a tracr RNA, and a tracr mate sequence, wherein the at least one guide sequence hybridizes with a target sequence. The expression of Cas9 may be inducible.

[0017] In one embodiment, the vector is configured to be conditional, whereby the vector targets only certain cell types. The vector may be a viral vector. The vector may be conditional by using a regulatory element that is cell or tissue specific. The regulatory element may be a promoter. The vector may be conditional by using a viral vector that infects a specific cell type. The vector may be any virus that efficiently targets cells of the central nervous system and does not illicit a strong immune reaction. The viral vector may be a lentivirus, an adenovirus, or an adeno associated virus (AAV). The virus envelope proteins may be chosen to cause the virus to have tropism towards a specific cell type. The vesicular stomatitis virus (VSV) envelope protein may be used to make a virus conditional.

[0018] The disease may be any nervous system disease where a model of disease exists or can be created. The screening method may be used to screen for modulators in Huntington's Disease, Alzheimer's disease, Parkinson's disease, and ALS. In preferred embodiments the disease is Huntington's Disease or Parkinson's Disease. The first mammal may be the R6/2 Huntington's disease model line.

[0019] In a second aspect, the present invention provides a method of treating a nervous system disease. The method may comprise activating expression of *Gpx6* in the central nervous system of a subject in need thereof suffering from the disease. The activation may be by a small molecule or compound. The small molecule or compound may be identified using biochemical and cell based assays. Additionally, protein therapeutics could be used to activate *Gpx6*. Treatment may be a single dose, multiple doses over a period of time, or doses on schedule for life. The schedule may be e.g., weekly, biweekly, every three weeks, monthly, bimonthly, every quarter year (every three months), every third of a year (every four months), every five months, twice yearly (every six months), every seven months, every eight months, every nine months, every ten months, every eleven months, annually or the like.

[0020] The method may comprise expressing *Gpx6* in the central nervous system of a subject in need thereof suffering from the disease. *Gpx6* may be expressed by introduction of a plasmid

by injection or by gene gun. *Gpx6* may also be introduced by viral vector such as AAV, adenovirus, or lentivirus.

[0021] The method may comprise introducing into a subject in need thereof suffering from the disease a CRISPR-Cas9 based system configured to target *Gpx6*. The CRISPR/Cas system may comprise a functional domain that activates transcription of the *Gpx6* gene. The functional domain may be an activator domain.

[0022] The disease may be any nervous system disease. The nervous system disease may be Huntington's Disease or Parkinson's Disease. Treating with a modulator by either effecting its expression or by introducing a vector to express the protein may not completely alleviate symptoms. Therefore, other drugs that specifically target the symptoms can be combined with that of a modulator. One may decrease the normal dose of the drug given due to the combination. The frequency of the drug may also be adjusted. The method may further comprise administering to a subject in need thereof suffering from the disease at least one of the drugs selected from the group consisting of Tetrabenazine, neuroleptics, benzodiazepines, amantadine, anti Parkinson's drugs, valproic acid, antioxidants, and Gpx mimetics. Central nervous system diseases are associated with oxidative stress, as well as, having neurological symptoms that lead to both mental and physical abnormalities. A combination therapy may be used to synergistically alleviate these symptoms. Antioxidants and Gpx mimetics may be used when a modulator involved in oxidative stress is identified.

[0023] In a third aspect, the present invention provides a method of determining a prognosis for a central nervous system disease comprising: obtaining a RNA sample from a patient suffering from a central nervous system disease; assaying the level of *Gpx6* gene expression; and comparing the levels of *Gpx6* gene expression to a control level determined by testing healthy subjects, wherein the prognosis is worse if *Gpx6* gene expression is lower than the control level. The method may further comprise assaying the level of *DARPP-32* gene expression; and comparing the levels of *DARPP-32* gene expression to a control level determined by testing healthy subjects, wherein the prognosis is worse if *DARPP-32* gene expression is lower than the control level.

[0024] In a fourth aspect, the present invention provides an antibody comprising a heavy chain and a light chain, wherein the antibody binds to an antigenic region of the Gpx6 protein comprising SEQ ID No: 1.

[0025] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0026] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as “comprises”, “comprised”, “comprising” and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean “includes”, “included”, “including”, and the like; and that terms such as “consisting essentially of” and “consists essentially of” have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0027] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0029] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, incorporated herein by reference wherein:

[0030] **Figure 1.** Illustrates gene expression changes associated with normal aging in cortical and striatal dopaminoceptive cell types. Venn diagram showing the number and overlap of statistically significant gene expression changes in dopamine receptor 1a (Drd1a)- or dopamine receptor 2 (Drd2)-expressing cortical or striatal neurons, based on a comparison of mice aged 6 weeks of age versus 2 years, 6 weeks of age. Statistically significant changes are defined as

genes displaying  $\geq 1.2$ -fold change and a Benjamini-Hochberg adjusted p-value from Welch's t test of  $\leq 0.05$ .

[0031] **Figure 2.** Illustrates the Synthetic lethal in the CNS (SLIC) screen. Top: Lentiviral genome-wide overexpression or knockdown libraries are injected into the striatum, such that each neuron or glial cell receives on average of one element (schematized by different colors). Lentivirus integrates into the cell's genome and expresses either a cDNA or shRNA. Bottom: After incubation *in vivo*, cells that have received a synthetic lethal hit die and the representation of these library elements are lost (an event that can be revealed by sequencing of all of the lentiviruses still present in the brain). When injections are performed in a paired fashion, comparing disease model mice to wild-type littermates, genes that cause synthetic lethality only in combination with a disease-causing mutation can be identified.

[0032] **Figure 3.** Illustrates the number of striatal cells transduced by the vesicular stomatitis virus G (VSV-G) coated lentivirus used in this study. EGFP cDNA-expressing lentivirus was injected into male mouse striatum 8 weeks of age and tissue was processed four days later for indirect immunofluorescent staining using antibodies directed toward GFP (marking transduced cells). By comparison of DAPI stained cells to EGFP-expressing cells, approximately 20% of cells in any rostrocaudal region of the striatum were transduced (EGFP positive). Based on a number of  $1.4 \times 10^6$  million striatal cells per animal (Fentress, Cowan et al., 1981), we thus calculate that the upper limit of transduction is  $2.8 \times 10^5$  striatal cells.

[0033] **Figure 4.** Illustrates striatal cell types infected by the vesicular stomatitis virus G (VSV-G) coated lentivirus used in this study. EGFP cDNA-expressing lentivirus was injected into male mouse striatum 8 weeks of age and tissue was processed four days later for indirect immunofluorescent staining using antibodies directed toward GFP (marking transduced cells), NeuN (neuronal marker), and GFAP (astrocyte marker). Based on immunofluorescent staining with these markers, approximately 83% of transduced cells are neurons, 14% are astrocytes, and 3% are unidentified cells.

[0034] **Figure 5A-5C.** Illustrates SLIC screening in mouse models of Huntington's disease. (A) Control small hairpin RNA (shRNA) representation in the striatum of wild-type animals, as determined by shRNA barcode sequencing, at 4 and 6 weeks after injection, each compared to a control 2 day time-point. A negative number reflects loss versus the control time-point. The positive control, a hairpin targeting the *Psmc2* gene product, would be expected to cause cell

death, leading to loss of its representation. Negative control shRNAs used (Table 9) had no known target in the genome. (B) shRNA barcode sequence representation at the first SLIC HD time-point. Graph represents  $\log_2$  fold changes in representation in the HD model at 4 weeks compared to the control 2-day time-point (R6/2 value, y axis), versus wild-type controls at the same two time-points (WT value, x axis). The positive control targeting the *Psmc2* gene product is not plotted for the purposes of scaling. Diagonal line represents equal representation ( $x=y$ ). Genes causing synthetic lethality are expected to be offset to the right of the diagonal in the bottom left quadrant of the graph. Gpx6 targeting shRNAs are denoted in red. (C) SLIC results for synthetic lethal hits that induce loss of representation, plotting % lentiviral element depletion seen in the HD model (R6/2) versus congenic wild-type animals at 4 weeks (left panel) and 6 weeks (right panel) of incubation. Controls are not represented. Gpx6 targeting shRNAs are denoted in red.

[0035] **Figure 6.** Illustrates that Gpx6 expression is down-regulated in the brains of Huntington's disease model mice. RNA was purified from the striatum of male R6/2 and control mice aged 8 weeks, and messenger RNA (mRNA) was converted to cDNA and used for quantitative PCR to measure Gpx6 mRNA abundance. Average cycle threshold values relative to *Eif4a2* ( $\Delta C_t$ ) are plotted with standard deviation. A higher  $\Delta C_t$  value (closer to 0) signifies higher abundance. A two-tailed unpaired t-test reveals a significance in difference between the means,  $p = 0.0002$ .

[0036] **Figure 7.** Illustrates Gpx6 mRNA expression across mouse brain regions. A cDNA panel representing 13 brain regions, as well as whole mouse brain, was used for quantitative PCR to measure Gpx6 mRNA abundance in adult mouse brain (10 weeks of age). Average cycle threshold values relative to actin ( $\Delta C_t$ ) are plotted with standard deviation. A lower  $\Delta C_t$  value signifies higher abundance.

[0037] **Figure 8.** Illustrates Gpx6 expression across normal aging. RNA was purified from the noted brain regions of male mice aged 1.5, 11, and 18 months, and messenger RNA (mRNA) was converted to cDNA and used for quantitative PCR to measure Gpx6 mRNA abundance. Average cycle threshold values relative to actin ( $\Delta C_t$ ) are plotted with standard deviation. A lower  $\Delta C_t$  value signifies higher abundance.

[0038] **Figure 9A-9B.** Illustrates the results of over-expressing Gpx6 in Huntington's disease model mice (A) Rescue of open field motor behavior in Huntington's disease model mice overexpressing Gpx6. Huntington's disease model mice (R6/2) or wild-type (WT) congenic controls were injected in the striatum bilaterally with Gpx6 or control (TRAP construct expressing) AAV9 virus at 6 weeks of age. After two weeks of recovery, motor function was assessed by open field assay. Average performance is plotted  $\pm$ SEM for each data point, reflecting total distance in cm travelled during a one-hour interval (R6/2+Gpx6 n= 10; R6/2+control n =10; WT+Gpx6 n=12; WT+control n=11). R6/2+Gpx6 vs. R6/2+control p value = 0.0165; WT+Gpx6 vs. WT+control p value = 0.7826 (no significance). (B) Increased DARPP-32 expression in Huntington's disease model mice overexpressing Gpx6. Huntington's disease model mice (R6/2) or wild-type (WT) congenic controls were unilaterally injected with control (TRAP construct; left hemisphere) or Gpx6 overexpressing (right hemisphere) AAV9 virus at 6 weeks of age. After two weeks of recovery, mice were sacrificed and brain tissue was processed for indirect immunofluorescent staining. Top panel: representative images of R6/2 mice injected with Gpx6 and control AAV9. Bottom panel: quantitation of images (mean pixel intensity across imaging field) from equivalent points in the dorsal striatum, p value = 0.0026. No significant difference between control and Gpx6-injected hemispheres was observed in wild-type congenic controls (data not shown). A.U. signifies arbitrary fluorescence units.

[0039] **Figure 10.** Illustrates locomotor effects of Gpx6 overexpression in a Parkinson's disease model mouse line. Mice overexpressing mutant alpha-synuclein protein "PD" or wild type littermates were injected with a Gpx6 overexpression virus at 6 weeks of age. Motor phenotypes were tested by open field assay for 60 minutes at approximately 7 months of age. At this age, PD model mice exhibit hyperactivity before progressing to hypoactivity at a later age. Gpx6 overexpression rescued the PD model phenotype at this age.

#### DETAILED DESCRIPTION OF THE INVENTION

[0040] The invention provides a method for identifying modulators of central nervous system diseases and for treating with agonists or antagonists of the modulators or with the modulators themselves. The invention also provides the use of the modulators in determining prognosis and diagnosis of a central nervous system disease and providing individualized or personalized treatment. The method may comprise: (a) stereotaxically administering to each of a



first and second mammal of the same species at least one vector containing a barcode and a nucleic acid molecule that is transcribed *in vivo*, wherein the first mammal is a model of a human disease and the second mammal is a normal control mammal not a model of a human disease, and wherein the nucleic acid molecule is associated with a gene; (b) harvesting genomic DNA from the first mammal and the second mammal; (c) identifying the barcodes from the harvested genomic DNA; and (d) comparing the barcode representation from the first mammal and the second mammal, whereby a differential barcode representation in the first mammal indicates that the gene associated with the nucleic acid molecule is a modulator of the disease. In one embodiment, modulators are determined by a loss of barcode in the disease model mouse when compared to the control mouse. In another embodiment, modulators are determined by a gain of barcode in the disease model mouse when compared to the control mouse.

[0041] Several further aspects of the invention relate to screening for modulators associated with a wide range of central nervous system diseases which are further described on the website of the National Institutes of Health (website at <http://rarediseases.info.nih.gov/gard/diseases-by-category/17/nervous-system-diseases>). The central nervous system diseases may include but are not limited to Alzheimer's Disease, Huntington's Disease and other Triplet Repeat Disorders (see Table A), amyotrophic lateral sclerosis (ALS), and Parkinson's disease.

[0042] Table A.

Trinucleotide repeat disorders				
Polyglutamine (PolyQ) Diseases				
Type	Gene		Normal PolyQ repeats	Pathogenic PolyQ repeats
DRPLA (Dentatorubropallidolusian atrophy)	ATN1 or DRPLA		6 - 35	49 - 88
HD (Huntington's disease)	HTT (Huntingtin)		6 - 35	36 - 250
SBMA (Spinobulbar muscular atrophy or Kennedy disease)	Androgen receptor on the X chromosome.		9 - 36	38 - 62
SCA1 (Spinocerebellar ataxia Type 1)	ATXN1		6 - 35	49 - 88
SCA2 (Spinocerebellar ataxia Type 2)	ATXN2		14 - 32	33 - 77
SCA3 (Spinocerebellar ataxia Type 3 or Machado-Joseph disease)	ATXN3		12 - 40	55 - 86

SCA6 (Spinocerebellar ataxia Type 6)	CACNA1A		4 - 18	21 - 30
SCA7 (Spinocerebellar ataxia Type 7)	ATXN7		7 - 17	38 - 120
SCA17 (Spinocerebellar ataxia Type 17)	TBP		25 - 42	47 - 63
<b>Non-Polyglutamine Diseases</b>				
<b>Type</b>	<b>Gene</b>	<b>Codon</b>	<b>Normal/wild type</b>	<b>Pathogenic</b>
FRAXA (Fragile X syndrome)	FMR1, on the X-chromosome	CGG	6 - 53	230+
FXTAS (Fragile X-associated tremor/ataxia syndrome)	FMR1, on the X-chromosome	CGG	6 - 53	55-200
FRAXE (Fragile XE mental retardation)	AFF2 or FMR2, on the X-chromosome	CCG	6 - 35	200+
FRDA (Friedreich's ataxia)	FXN or X25, (frataxin—reduced expression)	GAA	7 - 34	100+
DM (Myotonic dystrophy)	DMPK	CTG	5 - 37	50+
SCA8 (Spinocerebellar ataxia Type 8)	OSCA or SCA8	CTG	16 - 37	110 - 250
SCA12 (Spinocerebellar ataxia Type 12)	PPP2R2B or SCA12	nnn On 5' end	7 - 28	66 - 78

[0043] Additionally, the central nervous system diseases may include but are not limited to 2-methyl-3-hydroxybutyric aciduria, 2-methylbutyryl-CoA dehydrogenase deficiency, 22q11.2 deletion syndrome, 22q13.3 deletion syndrome, 3-alpha hydroxyacyl-CoA dehydrogenase deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, Aarskog syndrome, Aase-Smith syndrome, Abetalipoproteinemia, Absence of septum pellucidum, Acanthocytosis, Aceruloplasminemia, Acrocallosal syndrome, Schinzel type, Acrofacial dysostosis Rodriguez type, Acute cholinergic dysautonomia, Acute disseminated encephalomyelitis, Adenylosuccinase deficiency, Adie syndrome, Adrenomyeloneuropathy, Advanced sleep phase syndrome, familial, AGAT deficiency, Agnosia, Aicardi syndrome, Aicardi-Goutieres syndrome type 5, Albinism deafness syndrome, Alexander disease, Alopecia, Alpers syndrome, Alpha-ketoglutarate dehydrogenase deficiency, Alpha-mannosidosis type 1, Alpha-thalassemia x-linked intellectual disability syndrome, Alternating hemiplegia of childhood, Aminoacylase 1 deficiency, Amish infantile epilepsy syndrome, Amish lethal microcephaly, Amyloid neuropathy, Amyloidosis cerebral, Anaplastic ganglioglioma, Andermann syndrome, Andersen-Tawil syndrome, Anencephaly, Angioma hereditary neurocutaneous, Aniridia renal agenesis psychomotor

retardation, Apraxia, Arachnoid cysts, Arachnoiditis, Arthrogyrosis dysplasia, Aspartylglycosaminuria, Ataxia telangiectasia, Atelosteogenesis , Athabaskan brainstem dysgenesis, Atkin syndrome, Atypical Rett syndrome, Bannayan-Riley-Ruvalcaba syndrome, Barth syndrome, Basal ganglia disease, biotin-responsive, Basilar migraine, Battaglia Neri syndrome, Batten disease, Becker muscular dystrophy, Behcet's disease, Bell's palsy, Benign familial neonatal-infantile seizures, Benign rolandic epilepsy (BRE), Bethlem myopathy, Bilateral frontal polymicrogyria, Bilateral frontoparietal polymicrogyria, Bilateral generalized polymicrogyria, Bilateral parasagittal parieto-occipital polymicrogyria, Bilateral perisylvian polymicrogyria, Binswanger's disease, Bird headed dwarfism Montreal type, Bixler Christian Gorlin syndrome, Blepharospasm, Bobble-head doll syndrome, Borjeson-Forssman-Lehmann syndrome, Boucher Neuhauser syndrome, Bowen-Conradi syndrome, Branchial arch syndrome X-linked, Brody myopathy, Brown-Sequard syndrome, Brown-Vialetto-Van Laere syndrome, Bullous dystrophy hereditary macular type, C syndrome, C-like syndrome, CADASIL, CAHMR syndrome, Camptodactyly arthropathy coxa vara pericarditis syndrome, CANOMAD syndrome, Cantu syndrome, Cardiocranial syndrome, Cardiofaciocutaneous syndrome, Carney complex, Cataract anterior polar dominant, Cataract ataxia deafness, Catel Manzke syndrome, Caudal regression syndrome, Central core disease, Central neurocytoma, Central post-stroke pain, Cerebellar ataxia , Cerebellar degeneration, Cerebellar hypoplasia, Cerebellum agenesis hydrocephaly, Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy, Cerebral cavernous malformation, Cerebral dysgenesis neuropathy ichthyosis and palmoplantar keratoderma syndrome, Cerebral folate deficiency, Cerebral gigantism jaw cysts, Cerebral palsy, Cerebral sclerosis similar to Pelizaeus-Merzbacher disease, Cerebro-oculo-facio-skeletal syndrome, Cerebrospinal fluid leak, Cerebrotendinous xanthomatosis, Ceroid lipofuscinosis neuronal, Cervical hypertrichosis peripheral neuropathy, Chanarin-Dorfman syndrome, Charcot-Marie-Tooth disease , Chediak-Higashi syndrome, Chiari malformation, Choreoacanthocytosis, Choroid plexus carcinoma, Choroid plexus papilloma, Christianson syndrome, Chromosome 19q13.11 deletion syndrome, Chromosome 1p36 deletion syndrome, Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids, Chudley Rozdilsky syndrome, Cleft palate short stature vertebral anomalies, COACH syndrome, Cockayne syndrome , Coenzyme Q10 deficiency, Coffin-Lowry syndrome, Coffin-Siris syndrome, Cohen syndrome, Complex regional pain syndrome,

Congenital central hypoventilation syndrome, Congenital cytomegalovirus, Congenital disorder of glycosylation type 1B, Congenital disorder of glycosylation type 2C, Congenital fiber type disproportion, Congenital generalized lipodystrophy type 4, Congenital insensitivity to pain with anhidrosis, Congenital muscular dystrophy type 1A, Congenital myasthenic syndrome with episodic apnea, Congenital rubella, Convulsions benign familial infantile, Corneal hypesthesia familial, Cornelia de Lange syndrome, Corticobasal degeneration, Costello syndrome, Cowchock syndrome, Crane-Heise syndrome, Craniofrontonasal dysplasia, Craniopharyngioma, Craniotelencephalic dysplasia, Creutzfeldt-Jakob disease, Crisponi syndrome, Crome syndrome, Curry Jones syndrome, Cyprus facial neuromusculoskeletal syndrome, Cytomegalic inclusion disease, Dancing eyes-dancing feet syndrome, Dandy-Walker like malformation with atrioventricular septal defect, Danon disease, Dementia familial British, Dentatorubral-pallidoluysian atrophy, Dermatomyositis, Devic disease, Dihydropteridine reductase deficiency, Distal myopathy Markesbery-Griggs type, Distal myopathy with vocal cord weakness, Dopamine beta hydroxylase deficiency, Dravet syndrome, Duane syndrome, Dubowitz syndrome, Dwarfism, mental retardation and eye abnormality, Dykes Markes Harper syndrome, Dysautonomia like disorder, Dysequilibrium syndrome, Dyskeratosis congenita, Dyssynergia cerebellaris myoclonica, Dystonia , Early-onset ataxia with oculomotor apraxia and hypoalbuminemia, Emery-Dreifuss muscular dystrophy X-linked, Empty sella syndrome, Encephalitis lethargica, Encephalocraniocutaneous lipomatosis, Encephalomyopathy, Eosinophilic fasciitis, Epidermolysa bullosa simplex with muscular dystrophy, Epilepsy, Epiphyseal dysplasia hearing loss dysmorphism, Episodic ataxia with nystagmus, Erythromelalgia, Essential tremor, Fabry disease, Facial onset sensory and motor neuronopathy, Facioscapulohumeral muscular dystrophy, Fallot complex with severe mental and growth retardation, Familial amyloidosis, Finnish type, Familial congenital fourth cranial nerve palsy, Familial dysautonomia, Familial encephalopathy with neuroserpin inclusion bodies, Familial exudative vitreoretinopathy, Familial hemiplegic migraine, Familial idiopathic basal ganglia calcification, Familial transthyretin amyloidosis, Farber's disease, Fatal familial insomnia, Fatty acid hydroxylase-associated neurodegeneration, Fazio Londe syndrome, Febrile infection-related epilepsy syndrome, Feigenbaum Bergeron Richardson syndrome, Filippi syndrome, Fine-Lubinsky syndrome, Fitzsimmons Walson Mellor syndrome, Fitzsimmons-Guilbert syndrome, Floating-Harbor syndrome, Florid cemento-osseous dysplasia, Flynn Aird syndrome, Focal

dermal hypoplasia, Fountain syndrome, Fragile X syndrome, Fragile XE syndrome, Franek Bocker kahlen syndrome, Friedreich ataxia, Frontometaphyseal dysplasia, Frontotemporal dementia, Fryns syndrome, Fucosidosis, Fukuyama type muscular dystrophy, Fumarase deficiency, Galactosialidosis, GAPO syndrome, Gaucher disease type , Gemignani syndrome, Geniospasm, Genoa syndrome, Gerstmann syndrome, Gerstmann-Straussler-Scheinker disease, Giant axonal neuropathy, Gillespie syndrome, Glucose transporter type 1 deficiency syndrome, Glutaric acidemia , Glycogen storage disease, GM1 gangliosidosis , Goldberg-Shprintzen megacolon syndrome, Gomez Lopez Hernandez syndrome, Granulomatosis with polyangiitis (Wegener's), Griscelli syndrome type 1, Grubben de Cock Borghgraef syndrome, GTP cyclohydrolase I deficiency, Guanidinoacetate methyltransferase deficiency, Guillain-Barre syndrome, Gurrieri syndrome, Hamanishi Ueba Tsuji syndrome, Hansen's disease, Harding ataxia, Harrod Doman Keele syndrome, Hartnup disease, Hashimoto's encephalitis, Hemangioblastoma, Hemicrania continua, Hemiplegic migraine, Hennekam syndrome, Hereditary angiopathy with nephropathy aneurysms and muscle cramps syndrome, Hereditary endotheliopathy retinopathy nephropathy and stroke, Hereditary hemorrhagic telangiectasia, Hereditary hyperekplexia, Hereditary neuropathy with liability to pressure palsy, Hereditary sensory and autonomic neuropathy type 2, Hereditary sensory neuropathy type 1, Hereditary spastic paraplegia, Homocysteinemia due to MTHFR deficiency, Homocystinuria due to CBS deficiency, Hoyeraal Hreidarsson syndrome, HTLV-1 associated myelopathy/tropical spastic paraparesis, Huntington disease, Hyde Forster Mccarthy Berry syndrome, Hydranencephaly, Hydrocephalus due to congenital stenosis of aqueduct of sylvius, Hydroxykynureninuria, Hyperkalemic periodic paralysis, Hyperphenylalaninemia due to dehydratase deficiency, Hyperprolinemia, Hypertrophic neuropathy of Dejerine-Sottas, Hypogonadism alopecia diabetes mellitus mental retardation and extrapyramidal syndrome, Hypokalemic periodic paralysis, Hypomyelination and congenital cataract, Hypomyelination with atrophy of basal ganglia and cerebellum, Hypoparathyroidism-retardation-dysmorphism syndrome, Hypospadias mental retardation Goldblatt type, Hypothalamic hamartomas, Ichthyosis alopecia eclabion ectropion mental retardation, Idiopathic spinal cord herniation, Inclusion body myopathy, Incontinentia pigmenti, Infantile axonal neuropathy, Infantile convulsions and paroxysmal choreoathetosis, familial, Infantile myofibromatosis, Infantile onset spinocerebellar ataxia, Infantile Parkinsonism-dystonia, Infantile spasms broad thumbs, Inherited peripheral neuropathy,

Intellectual deficit, Internal carotid agenesis, Intraneural perineurioma, Isodicentric chromosome 15 syndrome, Johanson Blizzard syndrome, Johnson neuroectodermal syndrome, Joubert syndrome, Juberg Marsidi syndrome, Juvenile dermatomyositis, Juvenile primary lateral sclerosis, Kabuki syndrome, Kanzaki disease, Kapur Toriello syndrome, KBG syndrome, Kearns Sayre syndrome, Kennedy disease, Keutel syndrome, King Denborough syndrome, Kleine Levin syndrome, Klumpke paralysis, Kosztolanyi syndrome, Kuru, L-2-hydroxyglutaric aciduria, Laband syndrome, Lafora disease, Laing distal myopathy, Lambert Eaton myasthenic syndrome, LCHAD deficiency, Leigh syndrome, French Canadian type, Leisti Hollister Rimoin syndrome, Lennox-Gastaut syndrome, Lenz Majewski hyperostotic dwarfism, Lenz microphthalmia syndrome, Lesch Nyhan syndrome, Leukodystrophy with oligodontia, Leukodystrophy, dysmyelinating, and spastic paraparesis with or without dystonia, Levic Stefanovic Nikolic syndrome, Lhermitte-Duclos disease, Li-Fraumeni syndrome, Limb dystonia, Limb-girdle muscular dystrophy, Limited scleroderma, Lissencephaly, Localized hypertrophic neuropathy, Locked-in syndrome, Logopenic progressive aphasia, Lowe oculocerebrorenal syndrome, Lowry Maclean syndrome, Lujan Fryns syndrome, Mac Dermot Winter syndrome, Machado-Joseph disease, Macrogyria, pseudobulbar palsy and mental retardation, Macrothrombocytopenia progressive deafness, Mal de débarquement, Male pseudohermaphroditism intellectual disability syndrome, Verloes type, Malignant hyperthermia, Mannosidosis, beta A, lysosomal, Marchiafava Bignami disease, Marden-Walker syndrome, Marinesco-Sjogren syndrome, Martsolf syndrome, Maternally inherited Leigh syndrome, McDonough syndrome, McLeod neuroacanthocytosis syndrome, Meckel syndrome, Medrano Roldan syndrome, Medulloblastoma, Megalencephalic leukoencephalopathy with subcortical cysts, Mehes syndrome, Meier-Gorlin syndrome, Meige syndrome, Melnick-Needles syndrome, Meningioma, Meningioma, spinal, Menkes disease, Mental deficiency-epilepsy-endocrine disorders, Mental retardation, Meralgia paresthetica, Methionine adenosyltransferase deficiency, Methylcobalamin deficiency cbl G type, Microbrachycephaly ptosis cleft lip, Microcephalic osteodysplastic primordial dwarfism type 1, Microcephalic primordial dwarfism Toriello type, Microcephaly, Microphthalmia syndromic, Microscopic polyangiitis, Miller-Dieker syndrome, Miller-Fisher syndrome, Minicore myopathy with external ophthalmoplegia, Mitochondrial complex II deficiency, Mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes, Mitochondrial myopathy, Mitochondrial neurogastrointestinal encephalopathy syndrome,

Mitochondrial trifunctional protein deficiency, Mixed connective tissue disease, Miyoshi myopathy, Moebius syndrome, Molybdenum cofactor deficiency, Morse-Rawnsley-Sargent syndrome, Morvan's fibrillary chorea, Motor neuropathy peripheral with dysautonomia, Mousa Al din Al Nassar syndrome, Moyamoya disease, MPV17-related hepatocerebral mitochondrial DNA depletion syndrome, Mucopolysaccharidosis, Multifocal motor neuropathy, Multiple myeloma, Multiple sulfatase deficiency, Multiple system atrophy (MSA), Muscle eye brain disease, Muscular dystrophy white matter spongiosis, Muscular phosphorylase kinase deficiency, Myasthenia gravis, Myelocerebellar disorder, Myelomeningocele, Myhre syndrome, Myoclonic astatic epilepsy, Myoclonus, Myoglobinuria recurrent, Myopathy congenital multicore with external ophthalmoplegia, Myotonia congenita, Myotonic dystrophy, Nance-Horan syndrome, Narcolepsy, Native American myopathy, Nemaline myopathy 5, Neonatal adrenoleukodystrophy, Neonatal meningitis, Neonatal progeroid syndrome, Neu Laxova syndrome, Neuroaxonal dystrophy, infantile, Neuroblastoma, Neurocutaneous melanosis, Neurofaciodigitorenal syndrome, Neuroferritinopathy, Neurofibromatosis, Neuromyelitis optica spectrum disorder, Neuronal ceroid lipofuscinoses, Neuronal intranuclear inclusion disease, Neuropathy, Neuropathy, Neutral lipid storage disease with myopathy, Nevoid basal cell carcinoma syndrome, Nicolaides Baraitser syndrome, Niemann-Pick disease type B, Non 24 hour sleep wake disorder, Nondystrophic myotonia, Normokalemic periodic paralysis, Norrie disease, Northern Epilepsy, Occult spinal dysraphism, Oculocerebrocutaneous syndrome, Oculofaciocardiodental syndrome, Oculopharyngeal muscular dystrophy, Ohtahara syndrome, Okamoto syndrome, Oligoastrocytoma, Oliver syndrome, Olivopontocerebellar atrophy, Omphalocele cleft palate syndrome lethal, Optic atrophy 2, Ornithine transcarbamylase deficiency, Orofaciodigital syndrome, Osteopenia and sparse hair, Osteoporosis-pseudoglioma syndrome, Oto-palato-digital syndrome type 1, Ouvrier Billson syndrome, Pachygyria, Pallidopyramidal syndrome, Pallister W syndrome, Pallister-Killian mosaic syndrome, Pantothenate kinase-associated neurodegeneration, Paralysis agitans, juvenile, Paramyotonia congenital, Parenchymatous cortical degeneration of cerebellum, Paroxysmal hemicranias, Parsonage Turner syndrome, PEHO syndrome, Pelizaeus-Merzbacher disease, Pelizaeus-Merzbacher disease, late-onset type, Periventricular leukomalacia, Perry syndrome, Peters plus syndrome, Pfeiffer Mayer syndrome, Pfeiffer Palm Teller syndrome, PHACE syndrome, Phosphoglycerate kinase deficiency, Phosphoglycerate mutase deficiency, Photosensitive

epilepsy, Pick's disease, Pitt-Hopkins syndrome, POEMS syndrome, Poliomyelitis, Polyarteritis nodosa, Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, Polydactyly cleft lip palate psychomotor retardation, Polyglucosan body disease, adult, Polyneuropathy mental retardation acromicria premature menopause, Pontine tegmental cap dysplasia, Pontocerebellar hypoplasia, Post Polio syndrome, Posterior column ataxia, Potassium aggravated myotonia, PPM-X syndrome, Prader-Willi habitus, osteopenia, and camptodactyly, Primary amebic meningoencephalitis, Primary angiitis of the central nervous system, Primary basilar impression, Primary carnitine deficiency, Primary lateral sclerosis, Primary melanoma of the central nervous system, Primary progressive aphasia, Progressive bulbar palsy, Progressive hemifacial atrophy, Progressive non-fluent aphasia, Proteus syndrome, Proud Levine Carpenter syndrome, Pseudoaminopterin syndrome, Pseudoneonatal adrenoleukodystrophy, Pseudoprogeria syndrome, Pseudotrismy 13 syndrome, Pseudotumor cerebri, Pudendal Neuralgia, Pure autonomic failure, Pyridoxal 5'-phosphate-dependent epilepsy, Pyridoxine-dependent epilepsy, Pyruvate dehydrogenase phosphatase deficiency, Qazi Markouizos syndrome, Radiation induced brachial plexopathy, Rasmussen encephalitis, Reardon Wilson Cavanagh syndrome, Reducing body myopathy, Refsum disease, Refsum disease, infantile form, Renal dysplasia-limb defects syndrome, Renier Gabreels Jasper syndrome, Restless legs syndrome, Retinal vasculopathy with cerebral leukodystrophy, Rett syndrome, Richards-Rundle syndrome, Rigid spine syndrome, Ring chromosome, Rippling muscle disease, Roussy Levy syndrome, Ruvalcaba syndrome, Sacral defect with anterior meningocele, Salla disease, Sandhoff disease, Sarcoidosis, Say Barber Miller syndrome, Say Meyer syndrome, Scapuloperoneal syndrome, neurogenic, Kaeser type, SCARF syndrome, Schimke immunoosseous dysplasia, Schindler disease, type 1, Schinzel Giedion syndrome, Schisis association, Schizencephaly, Schwannomatosis, Schwartz Jampel syndrome type 1, Scott Bryant Graham syndrome, Seaver Cassidy syndrome, Seckel syndrome, Segawa syndrome, autosomal recessive, Semantic dementia, Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis, Sepiapterin reductase deficiency, Septo-optic dysplasia, SeSAME syndrome, Shapiro syndrome, Sharp syndrome, Short chain acyl CoA dehydrogenase deficiency, Shprintzen-Goldberg craniosynostosis syndrome, Sialidosis, Siderius X-linked mental retardation syndrome, Sideroblastic anemia and mitochondrial myopathy, Simpson-Golabi-Behmel syndrome, Single upper central incisor, Sjogren-Larsson syndrome, Slow-channel congenital myasthenic



syndrome, Smith-Lemli-Opitz syndrome type 1, Smith-Magenis syndrome, Sneddon syndrome, Snyder-Robinson syndrome, Sonoda syndrome, Spasmodic dysphonia, Spastic ataxia Charlevoix-Saguenay type, Spastic diplegia, Spastic paraplegia, Spina bifida occulta, Spinal muscular atrophy, Spinal shock, Spinocerebellar ataxia, Spinocerebellar degeneration and corneal dystrophy, Split hand urinary anomalies spina bifida, Spondyloepiphyseal dysplasia congenital, Status epilepticus, Steinfeld syndrome, Stratton-Garcia-Young syndrome, Striatonigral degeneration infantile, Sturge-Weber syndrome, Subacute sclerosing panencephalitis, Subcortical band heterotopia, Subependymoma, Succinic semialdehyde dehydrogenase deficiency, Susac syndrome, Symmetrical thalamic calcifications, Tangier disease, Tarlov cysts, Tay-Sachs disease, Tel Hashomer camptodactyly syndrome, Temporal epilepsy, familial, Temtamy syndrome, Thalamic degeneration symmetrical infantile, Thalamic degeneration, symmetric infantile, Thoracic outlet syndrome, Thyrotoxic periodic paralysis, Toriello Carey syndrome, Torsion dystonia with onset in infancy, Tourette syndrome, Transverse myelitis, Trichinosis, Trichorhinophalangeal syndrome type 2, Trigeminal neuralgia, Triose phosphate-isomerase deficiency, Triple A syndrome, Tuberos sclerosis, Tubular aggregate myopathy, Tyrosinemia type 1, Ullrich congenital muscular dystrophy, Unverricht-Lundborg disease, Van Benthem-Driessen-Hanveld syndrome, Van Den Bosch syndrome, Variant Creutzfeldt-Jakob disease, Vein of Galen aneurysm, Vici syndrome, Viljoen Kallis Voges syndrome, VLCAD deficiency, Vogt-Koyanagi-Harada syndrome, Von Hippel-Lindau disease, Walker-Warburg syndrome, Warburg micro syndrome, Weaver syndrome, Welander distal myopathy, Swedish type, Wernicke-Korsakoff syndrome, West syndrome, Westphal disease, Whispering dysphonia, Wieacker syndrome, Williams syndrome, Wilson disease, Wittwer syndrome, Wolf-Hirschhorn syndrome, Wolman disease, Worster Drought syndrome, Wrinkly skin syndrome, X-linked Charcot-Marie-Tooth disease type 5, X-linked creatine deficiency, X-linked myopathy with excessive autophagy, X-linked periventricular heterotopia, Young Hughes syndrome, Zechi Ceide syndrome, and Zellweger syndrome.

[0044] In one embodiment the disease is monogenic, affects defined cell populations in an age-dependent manner, and the mouse model displays minimal cell loss. This latter feature is particularly advantageous to the screening scheme, as synthetic lethal screens require a mild phenotype around which to screen for an enhanced phenotype.

[0045] The screening method may be used to identify modulators for any central nervous system diseases where an animal model is available. Several animal models have been described for the most prominent of the central nervous system diseases (Harvey et al., (2011) *J. Neural Transm.*; 118(1): 27-45; Ribeiro et al., (2013) *Rev Bras Psiquiatr.* 35 Suppl 2:S82-91). In some methods of the invention the organism or subject is a non-human eukaryote or a non-human animal or a non-human mammal. A non-human mammal may be for example a rodent (preferably a mouse or a rat), an ungulate, or a primate. In a preferred embodiment, the animal model is a mouse.

[0046] In another embodiment the animal model is a Huntington's disease (HD) model line. Mouse models have been created with CAG repeats of different lengths that have an HD phenotype: R6/1 with 116 repeats, R6/2 with 144 repeats and R6/5 with a wider spectrum of repeats. R6/2 mice have been studied most and show choreiform-like movements, involuntary stereotypic movements, tremor, epileptic seizures and premature death (Mangiarini et al., (1996) *Cell*, 87:493-506). In R6/2 mice the age of onset is 9–11 weeks and the age of death is 10–13 weeks. R6/2 mice have huntingtin aggregates in the nucleus of neurons seen prior to developing a neurological phenotype (Davies et al., (1997) *Cell.*, 90:537–548). Also, the mRNA for type 1 metabotropic glutamate receptors and for D1 dopamine receptors is already reduced at the age of 4 weeks (Cha et al., (1998) *Proc Natl Acad Sci USA*, 95:6480–6485). A transgenic rat model of HD, with a mutated huntingtin gene containing 51 CAG repeats, expresses adult-onset neurological phenotypes, cognitive impairments, progressive motor dysfunction and neuronal nuclear inclusions in the brain (von Horsten et al., (2003) *Hum Mol Genet.*, 12:617-624). The transgenic rats have a late onset of phenotype and they die between 15 and 24 months. Transgenic HD rats have an age and genotype dependent deterioration of psychomotor performance and choreiform symptoms (Cao et al., (2006) *Behav Brain Res.*, 170:257-261). Recently, HD was modeled in the rhesus macaque with a lentiviral vector (Cai et al., (2008) *Neurodegener Dis.*, 5:359–366). Yang et al. injected rhesus oocytes with lentivirus expressing exon 2 of the human huntingtin gene with 84 CAG repeats and five transgenic monkeys carrying mutant huntingtin were produced (Yang et al., (2008) *Proc Natl Acad Sci USA.*, 105:7070-7075). The monkeys showed the main features of HD disease including nuclear inclusions, neuropil aggregates and a behavioral phenotype but all of them died at an early stage of life. In a

preferred embodiment the mouse model is the R6/2 Huntington's disease model line (Mangiarini et al., (1996) *Cell*, 87:493-506).

[0047] In another embodiment the methods are used to identify modulators of Alzheimer's disease (AD). Alzheimer's disease is the most prevalent of neurodegenerative diseases that causes progressive memory loss and dementia in affected patients. Diagnosis of AD occurs post-mortem by confirming the presence of neurofibrillary tangles (NFT) and amyloid plaques which are found in the several brain regions including the subiculum and entorhinal cortex. The NFT are intraneuronal microtubule bundles containing hyperphosphorylated forms of microtubule associated protein tau (MAPT). The amyloid plaques are extracellular deposits primarily consisting of the amyloid  $\beta$  peptide. To date, 16 genes or loci have been identified for AD (OMIM 104300). The presence of NFTs in post-mortem brain is one of the defining pathologies of AD. However, there is no direct correlation between the number of cortical plaques and cognitive deficit in AD patients, and many individuals have amyloid plaques without cognitive impairment or dementia (Duyckaerts et al., (2009) *Acta Neuropathol.*, 118:5-36). Moreover, the amount and the topography of the senile plaques are not correlated with the severity of dementia, and the amyloid deposition seems to remain stable during the progression of the disease (Jack et al., (2010) *Lancet Neurol.*, 9:119-28). As such, in one embodiment, Alzheimer's disease is screened for modulators that can be used for diagnosis and treatment. There have been several transgenic mice generated based on mutations in the human MAPT gene that have provided clear evidence for mutant tau in NFT pathology and dementia (McGowan et al., (2006) *Trends Genet.*, 22:281-289). None of the transgenic rodent models based on single gene mutations have been able to fully recapitulate the features of AD. Combinations of transgenes have provided novel transgenic models that have a progressive pathology with behavioral deficits. Triple transgenic mice (3xTg-AD) have been produced and progressively develop synaptic dysfunction, APP-containing plaques and NFTs (Oddo et al., (2003) *Neurobiol Aging*, 24:1063-1070). The 3xTg-AD mouse has thus been the most widely used model of AD for evaluating potential therapies, examining environmental vulnerabilities and studying disease mechanism (Gimenez-Llort et al., (2007) *Neurosci Biobehav Rev.*, 31:125-147; Foy et al., (2008) *J Alzheimers Dis.*, 15:589-603). In addition to mouse models based on mutations found in human genes, there are non-transgenic models of AD in the rat, rabbit, dog and primate that offer the ability to conduct complementary studies for the evaluation of therapeutics and the understanding of disease mechanisms

(Woodruff-Pak, (2008) *J Alzheimers Dis.*, 15:507–521). In a preferred embodiment, the 3xTg-AD mouse is used with the screening methods.

[0048] In another embodiment the methods are used to identify modulator's of amyotrophic lateral sclerosis (ALS). Amyotrophic lateral sclerosis is a neurodegenerative disease that results from the progressive loss of motor neurons in brain and spinal cord. Onset of disease typically occurs in middle adulthood but forms with juvenile onset also occur. Symptoms include asymmetrical muscle weakness and muscle fasciculations. The disease progresses rapidly after onset leading to paralysis and eventually death within 5 years. The first gene associated with ALS was the superoxide dismutase-1 (SOD1) gene encoding an enzyme capable of inactivating superoxide radicals (Rosen et al., (1993) *Nature*, 362:59–62). Gurney et al. reported that mice over-expressing a human SOD1 allele containing a G93A substitution developed spinal cord motor neuron loss and related paralysis (Gurney et al., (1994) *Science*, 264:1772-1775). Following that initial study with the G93A variant, 13 additional transgenic mice have been made that produced a broad range of outcomes but all exhibit some characteristics of the disease (Ripps et al., (1995) *Proc Natl Acad Sci USA*, 92:689–693; Wong et al., (1995) *Neuron*, 14:1105-1116; Bruijn et al., (1997) *Neuron*, 18:327-338; Wang et al., (2002) *Neurobiol Dis.*, 10:128–138, (2003) *Hum Mol Genet.*, 12:2753–2764, (2005) *Hum Mol Genet.*, 14:2335–2347; Tobisawa et al., (2003) *Biochem Biophys Res Commun.*, 303:496–503; Jonsson et al., (2005) *Brain*, 127:73–88 (2004), *J Neuropathol Exp Neurol.*, 65:1126–1136 (2006); Chang-Hong et al., *Exp Neurol.*, 194:203-211; Watanabe et al., (2005) *Brain Res Mol Brain Res.*, 135:12-20; Deng et al., (2006) *Proc Natl Acad Sci USA*, 103:7142–7147). The *SOD1* animal collection has produced several therapeutic strategies (e.g. arimoclomol, ceftriaxone, IGF-1, HDAC inhibitors) that are now in clinical trials. In a preferred embodiment, a G93A mouse model is used to screen for modulators.

[0049] In another embodiment the methods are used to identify modulator's of Parkinson's disease (PD). Parkinson's disease is a slow, progressive neurodegenerative disorder that is characterized pathologically by the loss of dopaminergic neurons in the pars compacta of the substantia nigra. There currently is no mouse model for Parkinson's disease based on a mutation. For example, even though the gene is linked to the disease, overexpressing of human  $\alpha$ -synuclein or its mutated forms in transgenic mice is not sufficient to cause a complete Parkinsonian phenotype. In one embodiment this mouse is used to screen for modulators. In other

embodiments, mouse knockouts for the Park genes are used. The so-called neurotoxin-based models of PD are the most effective in reproducing irreversible dopaminergic neuron death and striatal dopamine deficit in nonhuman primates and rodents. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), 6-OHDA (6-hydroxy-dopamine), and rotenone are so far the most widely used compounds. They are particularly attractive for inducing cytotoxicity by oxidative stress mechanisms, as brain from PD patients show decreased levels of reduced glutathione and oxidative modifications to DNA, lipids, and proteins (Pearce et al., (1997) *J Neural Transm.*, 104:661-77; Floor et al., (1998) *J Neurochem.*, 70:268-75). Interestingly, MPTP was accidentally discovered during the investigations of the potential factors that led young addicts to develop PD-like symptoms. MPTP was found to be the heroin contaminant responsible for parkinsonism in these subjects (Ribeiro et al., (2013) *Rev Bras Psiquiatr.* 35 Suppl 2:S82-91). In a preferred embodiment, the neurotoxin based models are used to screen for modulators.

[0050] Among vectors that may be used in the practice of the invention, integration in the host genome of a central nervous system cell is possible with retrovirus gene transfer methods, often resulting in long term expression of the inserted transgene. In a preferred embodiment the retrovirus is a lentivirus. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues. The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. A retrovirus can also be engineered to allow for conditional expression of the inserted transgene, such that only certain cell types are infected by the lentivirus. Additionally, cell type specific promoters can be used to target expression in specific cell types. Lentiviral vectors are retroviral vectors (and hence both lentiviral and retroviral vectors may be used in the practice of the invention). Moreover, lentiviral vectors are preferred as they are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system may therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the desired nucleic acid into the target cell to provide permanent expression. Widely used retroviral vectors that may be used in the practice of the invention include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and

combinations thereof (see, e.g., Buchscher et al., (1992) J. Virol. 66:2731-2739; Johann et al., (1992) J. Virol. 66:1635-1640; Sommnerfelt et al., (1990) Virol. 176:58-59; Wilson et al., (1998) J. Virol. 63:2374-2378; Miller et al., (1991) J. Virol. 65:2220-2224; PCT/US94/05700).

[0051] Also useful in the practice of the invention is a minimal non-primate lentiviral vector, such as a lentiviral vector based on the equine infectious anemia virus (EIAV) (see, e.g., Balagaan, (2006) J Gene Med; 8: 275 – 285, Published online 21 November 2005 in Wiley InterScience ([www.interscience.wiley.com](http://www.interscience.wiley.com)). DOI: 10.1002/jgm.845). The vectors may have cytomegalovirus (CMV) promoter driving expression of the target gene. Accordingly, the invention contemplates amongst vector(s) useful in the practice of the invention: viral vectors, including retroviral vectors and lentiviral vectors. In a preferred embodiment lentiviral vectors are used to insert short hairpin RNAs (shRNAs), seeking genes that, when knocked down, would enhance mutant huntingtin toxicity. In another preferred embodiment lentiviral vectors are used to insert cDNA, seeking genes that, when overexpressed, would enhance mutant huntingtin toxicity.

[0052] Also useful in the practice of the invention is an adenovirus vector. One advantage is the ability of recombinant adenoviruses to efficiently transfer and express recombinant genes in a variety of mammalian cells and tissues *in vitro* and *in vivo*, resulting in the high expression of the transferred nucleic acids. Further, the ability to productively infect quiescent cells, expands the utility of recombinant adenoviral libraries. In addition, high expression levels ensure that the products of the nucleic acids will be expressed to sufficient levels to screen for changes in viability of infected cells (see e.g., U.S. Patent No. 7,029,848, hereby incorporated by reference). In addition libraries can utilize adeno associated virus as the vector, described herein.

[0053] Genetic screens, for example, for lethal events, can be carried out in a 96-well format where each well contains isolated cells and a different shRNA, cDNA, or CRISPR/Cas system encoding viral vector. However, this method cannot be performed *in vivo*. In another embodiment, a DNA barcoding strategy can be used *in vivo* with a pooled library of viral vectors. In one embodiment the viral vector can be identified by the barcode.

[0054] The term “barcode” as used herein, refers to any unique, non-naturally occurring, nucleic acid sequence that may be used to identify the originating source of a nucleic acid fragment. Such barcodes may be sequences including but not limited to, TTGAGCCT, AGTTGCTT, CCAGTTAG, ACCAACTG, GTATAACA or CAGGAGCC. Although it is not

necessary to understand the mechanism of an invention, it is believed that the barcode sequence provides a high-quality individual read of a barcode associated with a viral vector, shRNA, or cDNA such that multiple species can be sequenced together.

[0055] DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine classification but to identify an unknown sample in terms of a known classification. Kress et al., "Use of DNA barcodes to identify flowering plants" *Proc. Natl. Acad. Sci. U.S.A.* 102(23):8369-8374 (2005). Barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated. Koch H., "Combining morphology and DNA barcoding resolves the taxonomy of Western Malagasy *Liotrigona* Moure, 1961" *African Invertebrates* 51(2): 413-421 (2010); and Seberg et al., "How many loci does it take to DNA barcode a crocus?" *PLoS One* 4(2):e4598 (2009). Barcoding has been used, for example, for identifying plant leaves even when flowers or fruit are not available, identifying the diet of an animal based on stomach contents or feces, and/or identifying products in commerce (for example, herbal supplements or wood). Soininen et al., "Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures" *Frontiers in Zoology* 6:16 (2009).

[0056] It has been suggested that a desirable locus for DNA barcoding should be standardized so that large databases of sequences for that locus can be developed. Most of the taxa of interest have loci that are sequencable without species-specific PCR primers. CBOL Plant Working Group, "A DNA barcode for land plants" *PNAS* 106(31):12794-12797 (2009). Further, these putative barcode loci are believed short enough to be easily sequenced with current technology. Kress et al., "DNA barcodes: Genes, genomics, and bioinformatics" *PNAS* 105(8):2761-2762 (2008). Consequently, these loci would provide a large variation between species in combination with a relatively small amount of variation within a species. Lahaye et al., "DNA barcoding the floras of biodiversity hotspots" *Proc Natl Acad Sci USA* 105(8):2923-2928 (2008).

[0057] DNA barcoding is based on a relatively simple concept. For example, most eukaryote cells contain mitochondria, and mitochondrial DNA (mtDNA) has a relatively fast mutation rate, which results in significant variation in mtDNA sequences between species and, in principle, a

comparatively small variance within species. A 648-bp region of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene was proposed as a potential 'barcode'. As of 2009, databases of CO1 sequences included at least 620,000 specimens from over 58,000 species of animals, larger than databases available for any other gene. Ausubel, J., "A botanical microscope" Proceedings of the National Academy of Sciences 106(31):12569 (2009).

[0058] Software for DNA barcoding requires integration of a field information management system (FIMS), laboratory information management system (LIMS), sequence analysis tools, workflow tracking to connect field data and laboratory data, database submission tools and pipeline automation for scaling up to eco-system scale projects. Geneious Pro can be used for the sequence analysis components, and the two plugins made freely available through the Moorea Biocode Project, the Biocode LIMS and Genbank Submission plugins handle integration with the FIMS, the LIMS, workflow tracking and database submission.

[0059] Additionally other barcoding designs and tools have been described (see e.g., Birrell et al., (2001) Proc. Natl Acad. Sci. USA 98, 12608–12613; Giaever, et al., (2002) Nature 418, 387–391; Winzeler et al., (1999) Science 285, 901–906; and Xu et al., (2009) Proc Natl Acad Sci U S A. Feb 17;106(7):2289-94).

[0060] An advantage of this invention is that one neuron in a brain region is used as a genetic screening vehicle, as opposed to one mouse being used as a screening vehicle. Additionally, many modulators of disease outcome can be isolated in a single experiment in contrast to single genes. A modulator is a gene that effects phenotype progression in a disease (disease outcome) (e.g., see example 3). In one embodiment the upper limit of elements that can be screened are shRNA's targeting whole genomes including non-coding RNA's. In one embodiment the upper limit of elements that can be screened are cDNA's expressing genes encoded within whole genomes. In one embodiment cDNA's expressing genes that are known biomarkers of oxidative stress are screened and in another embodiment these genes are targeted by shRNA (see e.g., BOSS (NIEHS), <http://www.niehs.nih.gov/research/resources/databases/bosstudy/>). In one embodiment viral genome-wide overexpression or knockdown libraries are injected into a section of the brain of a mammal. In another embodiment viral genome-wide overexpression or knockdown libraries are injected into the striatum of a mammal, such that each neuron or glial cell receives on average of one element. In this embodiment each virus expresses either a cDNA or shRNA. Each cDNA expresses a gene that potentially modulates disease outcome, while each



shRNA causes repression of a gene that potentially modulates disease outcome. In one embodiment  $2.8 \times 10^5$  striatal cells are targeted per mouse, wherein over 80% of viral-transduced cells are neurons. In other mammals the number of cells targeted may be dependent on the size of the brain of the mammal. After incubation *in vivo*, cells that receive a synthetic lethal hit die and the representation of these library elements are lost. When injections are performed in a paired fashion, modulator's can be identified by comparing disease model mammals to wild-type littermates. Genes that cause synthetic lethality only in combination with a disease-causing mutation can be identified to be a modulator of disease. In contrast, in studies using mouse knockouts, a single gene in the entire mouse or cell type is deactivated.

[0061] In another embodiment a protein associated with oxidative stress is found to be a modulator of a central nervous system disease (see Example 2). There are two main families of proteins that detoxify peroxides (Day BJ (2009) *Biochemical pharmacology* 77(3):285-296). Superoxide dismutases (SOD) and catalase are metalloproteins that catalyze "dismutation" reactions. Another class of endogenous catalytic  $H_2O_2$  scavengers is the selenium-containing peroxidases. This is a broad group of enzymes that utilize  $H_2O_2$  as a substrate along with an endogenous source of reducing equivalence. One of the best studied families of peroxidases are the glutathione peroxidases (GPx). The glutathione peroxidase family includes the eight known glutathione peroxidases (Gpx1-8) in humans. Mammalian Gpx1, Gpx2, Gpx3, and Gpx4 have been shown to be selenium-containing enzymes, whereas Gpx6 is a selenoprotein in humans with cysteine-containing homologues in rodents. Several existing studies discuss the observation that selenocysteine-containing enzymes are typically 100 to 1000-fold more active than corresponding mutants where selenocysteine (Sec) is replaced with cysteine (Cys) (Shchedrina et al., (2007) *Proc Natl Acad Sci U S A.* 104(35): 13919–13924). This follows evidence that Sec is a more efficient redox catalyst than Cys. Thus, changing an enzyme's Sec to a Cys results in lower activity. In the case of some enzymes, changing their endogenous Cys to Sec, and adding a selenocysteine insertion sequence (SECIS) element, makes them more active in almost every case. The SECIS element is an RNA element around 60 nucleotides in length that adopts a stem-loop structure and directs the cell to translate UGA codons as selenocysteines. Adding a SECIS element may change enzyme activity. Thus, Cys containing enzymes might have different activity and substrate specificity. For example replacing Cys with Sec in MsrB2 and B3 led to inability to regenerate active enzymes by the natural electron donor. According to Kryukov et al.,

(2003) Science; 300(5624): 1439-43, Gpx6 is a close homologue of Gpx3, and the rat and mouse orthologs of Gpx6 contain Cys instead of Sec as is found in the human protein. They also note a lack of a functional SECIS unit in rodent Gpx6. Human Gpx6 is 72% homologous to mouse Gpx6. Therefore, in one embodiment the mouse homologue of a peroxidase protein is used in humans as a modulator of disease. In another embodiment a modulator that is a peroxidase protein can be mutated to contain a Cys instead of Sec or vice versa.

[0062] Studies have shown that Gpx6 levels correlate with dopamine levels in the brain, signifying that this gene may have relevance to other diseases linked to dopamine, including Parkinson's disease. Furthermore, Gpx6 levels correlate with aging (see Example 1). The other peroxidases, may also be modulators of central nervous system diseases, however the expression of these proteins do not show the same correlation as Gpx6.

[0063] In another embodiment a modulator may be involved in the regulation of dopamine signalling. Dopamine is a monoamine neurotransmitter that exerts its action on neuronal circuitry via dopamine receptors. As dopaminergic innervations are most prominent in the brain, dopaminergic dysfunction can critically affect vital central nervous system (CNS) functions, ranging from voluntary movement, feeding, reward, affect, to sleep, attention, working memory and learning (Carlsson, Beaulieu). Dysregulation of dopaminergic neurotransmission has been associated with multiple neurological and psychiatric conditions such as Parkinson's disease, Huntington's disease, attention deficit hyperactivity disorder (ADHD), mood disorders and schizophrenia (Carlsson, Ganetdinov and Caron), as well as various somatic disorders such as hypertension and kidney dysfunction (Missale, Beaulieu, Pharmacol. Rev. 2011, 63, 182).

[0064] In yet another aspect of the invention, the modulators of disease identified by the screening methods is used to treat a disease of the central nervous system by impeding phenotype progression of the disease. In one embodiment an agonist or antagonist of the biologic activity of the modulator is used to increase or decrease the activity of the modulator to improve disease outcome. The agonist or antagonist may be a small molecule or protein based therapeutic. Biochemical and cell based *in vitro* assays can be used to screen for the agonist or antagonist. The modulator can be purified or partially purified from cell extracts containing endogenous protein. This is advantageous in that the purified modulator includes its native post translational modifications and if it is part of a multiprotein complex, those associated proteins are copurified. Recombinant protein can also be expressed in mammalian cell culture, insect

cells, bacteria, or yeast. This is advantageous in that the modulator can be tagged, facilitating purification. Such tags include, for example, hexahistidine tags, HA, MYC, and Flag. Recombinant protein can be generated using a DNA vector. Most preferably a plasmid encoding the protein sequence of the modulator is used. The plasmid contains functional elements required for its amplification in prokaryotic cells. The plasmid may contain elements required for the modulator gene to be incorporated into a virus. The plasmid may contain elements that allow expression of the gene in mammalian cells, such as a mammalian promoter. The plasmid may also contain elements for expression in insect or prokaryotic cells. Advantages of insect cells are high protein expression and post translational modifications associated with eukaryotic cells. In one embodiment the modulator protein is used in an *in vitro* assay that recapitulates its biological activity. In one embodiment Gpx6 peroxidase activity is reconstituted *in vitro*. Compounds or molecules are incubated at their effective concentrations in the *in vitro* reconstituted assay with the modulator to test effects on biological activity. In another embodiment, compounds or molecules are tested in cell based assays. In one embodiment reporter genes specific to a modulator can be incorporated into a mammalian cell. In one embodiment promoters of genes up or down regulated during oxidative stress could be incorporated into a reporter construct. The reporter construct may express a marker such as luciferase or GFP. Small molecules that activate Gpx6 activity in the presence of oxidative stress may be screened by assaying for the reporter expression. The modulator may also be overexpressed in such a cell based assay. In another embodiment a therapeutic molecule that activates or represses the expression of the modulator can be used to treat the disease. A cell based assay where a reporter gene is operably linked to the promoter of the modulator can be used. In a specific embodiment the Gpx6 promoter is used.

[0065] Many compound or small molecule libraries exist and can be used to screen for agonists and antagonists. Additionally, libraries can be selected, constructed, or designed specifically for a modulator. In one embodiment agonists or antagonists of modulators can be screened using, for example, the NIH Clinical Collections (see, <http://www.nihclinicalcollection.com/>). The Clinical Collection and NIH Clinical Collection 2 are plated arrays of 446 and 281, respectively, small molecules that have a history of use in human clinical trials. In another embodiment collections of FDA approved drugs are assayed. Advantages of these collections are that the clinically tested compounds are highly drug-like

with known safety profiles. Additionally, agonists or antagonists can be modified based on known structures of the modulator and the small molecules.

[0066] In another embodiment molecules based on a modulator involved in oxidative stress can be used to treat the disease. The molecule may be a Gpx or peroxidase mimetic, catalase mimetic, or superoxide dismutase (SOD) mimetic (see e.g., Day BJ (2009) *Biochemical pharmacology* 77(3):285-296). Gpx mimetics can be classified in three major categories: (i) cyclic selenenyl amides having a Se-N bond, (ii) diaryl diselenides, and (iii) aromatic or aliphatic monoselenides. Additionally, small molecules, such as the antioxidant ebselen, that acts as a glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase mimic could be used to treat a central nervous system disease. Ebselen has been shown to substantially reduce gray and white matter damage and neurological deficit associated with transient ischemia (Imai et al., (2001) *Stroke; a journal of cerebral circulation* 32(9):2149-2154). In other embodiments, drugs used to treat strokes are used to effect a modulator of disease. Molecules such as the antioxidant Coenzyme Q10 may also be used to treat a nervous system disease. In one embodiment the small molecules are administered to pre-symptomatic populations.

[0067] In another embodiment a protein based therapeutic may be an agonist or antagonist of a modulator. In one embodiment the therapeutic protein is an antibody or antigen binding fragment of an antibody. In one embodiment the antibody or antigen binding fragment may bind to an inhibitor of the modulator. In a preferred embodiment the antibody is humanized, chimeric, or fully humanized.

[0068] In another embodiment the modulator is introduced into a subject in need thereof to treat a central nervous system disease. Treatment may include over-expressing or repressing the modulator in the cells of patient in need thereof effected by the disease. In a more specific embodiment a vector could be used to introduce a nucleic acid that encodes the modulator (see Example 3). In one embodiment, the modulator is introduced by viral delivery. The nucleic acids encoding modulators discovered by the screening method can be delivered using adeno associated virus (AAV), lentivirus, adenovirus or other viral vector types, or combinations thereof. Plasmids that can be used for adeno associated virus (AAV), adenovirus, and lentivirus delivery have been described previously (see e.g., U.S. Patent Nos. 6,955,808 and 6,943,019, and U.S. Patent application No. 20080254008, hereby incorporated by reference).

[0069] In terms of *in vivo* delivery, AAV is advantageous over other viral vectors due to low toxicity and low probability of causing insertional mutagenesis because it doesn't integrate into the host genome. AAV has a packaging limit of 4.5 or 4.75 Kb. Constructs larger than 4.5 or 4.75 Kb result in significantly reduced virus production. There are many promoters that can be used to drive nucleic acid molecule expression. AAV ITR can serve as a promoter and is advantageous for eliminating the need for an additional promoter element. For ubiquitous expression, the following promoters can be used: CMV, CAG, CBh, PGK, SV40, Ferritin heavy or light chains, etc. For brain expression, the following promoters can be used: SynapsinI for all neurons, CaMKIIalpha for excitatory neurons, GAD67 or GAD65 or VGAT for GABAergic neurons, etc. Promoters used to drive RNA can include: Pol III promoters such as U6 or H1. The use of a Pol II promoter and intronic cassettes can be used to express guide RNA (gRNA).

[0070] As to AAV, the AAV can be AAV1, AAV2, AAV5 or any combination thereof. One can select the AAV with regard to the cells to be targeted; e.g., one can select AAV serotypes 1, 2, 5 or a hybrid capsid AAV1, AAV2, AAV5 or any combination thereof for targeting brain or neuronal cells; and one can select AAV4 for targeting cardiac tissue. AAV8 is useful for delivery to the liver. The above promoters and vectors are preferred individually.

[0071] The virus may be delivered to the patient in need thereof in any way that allows the virus to contact the target cells in which delivery of the gene of interest is desired. Various means of delivery are described herein, and further discussed in this section. In some embodiments, the viral vector is delivered to the tissue of interest by, for example, an intramuscular or stereotaxic injection, while other times the viral delivery is via intravenous, transdermal, intranasal, oral, mucosal, or other delivery methods. In the provided method, the viral vector can be administered systemically. Such delivery may be either via a single dose, or multiple doses. One skilled in the art understands that the actual dosage to be delivered herein may vary greatly depending upon a variety of factors, such as the vector chosen, the target cell, organism, or tissue, the general condition of the subject to be treated, the degree of transformation/modification sought, the administration route, the administration mode, administration timing, the type of transformation/modification sought, etc.

[0072] In preferred embodiments, a suitable amount of virus is introduced into a patient in need thereof directly (*in vivo*), for example through injection into the body. In one such

embodiment, the viral particles are injected directly into the patient's brain, for example, intracranial injection using stereotaxic coordinates may be used to deliver virus to the brain.

[0073] Such a delivery may further contain, for example, a carrier (water, saline, ethanol, glycerol, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, etc.), a diluent, a pharmaceutically-acceptable carrier (e.g., phosphate-buffered saline or Hank's Balanced Salt Solution), a pharmaceutically-acceptable excipient, and/or other compounds known in the art. Such a dosage formulation is readily ascertainable by one skilled in the art. The dosage may further contain one or more pharmaceutically acceptable salts such as, for example, a mineral acid salt such as a hydrochloride, a hydrobromide, a phosphate, a sulfate, etc.; and the salts of organic acids such as acetates, propionates, malonates, benzoates, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, gels or gelling materials, flavorings, colorants, microspheres, polymers, suspension agents, etc. may also be present herein. In addition, one or more other conventional pharmaceutical ingredients, such as preservatives, humectants, suspending agents, surfactants, antioxidants, anticaking agents, fillers, chelating agents, coating agents, chemical stabilizers, etc. may also be present, especially if the dosage form is a reconstitutable form. Suitable exemplary ingredients include microcrystalline cellulose, carboxymethylcellulose sodium, polysorbate 80, phenylethyl alcohol, chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, parachlorophenol, gelatin, albumin and a combination thereof. A thorough discussion of pharmaceutically acceptable excipients is available in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Pub. Co., N.J. 1991) which is incorporated by reference herein.

[0074] In an embodiment herein the delivery is via an adenovirus, which may be at a single booster dose containing at least  $1 \times 10^5$  particles (also referred to as particle units, pu) of adenoviral vector. In an embodiment herein, the dose preferably is at least about  $1 \times 10^6$  particles (for example, about  $1 \times 10^6$ - $1 \times 10^{12}$  particles), more preferably at least about  $1 \times 10^7$  particles, more preferably at least about  $1 \times 10^8$  particles (e.g., about  $1 \times 10^8$ - $1 \times 10^{11}$  particles or about  $1 \times 10^8$ - $1 \times 10^{12}$  particles), and most preferably at least about  $1 \times 10^9$  particles (e.g., about  $1 \times 10^9$ - $1 \times 10^{10}$  particles or about  $1 \times 10^9$ - $1 \times 10^{12}$  particles), or even at least about  $1 \times 10^{10}$  particles (e.g., about  $1 \times 10^{10}$ - $1 \times 10^{12}$  particles) of the adenoviral vector. Alternatively, the dose comprises no more than about  $1 \times 10^{14}$  particles, preferably no more than about  $1 \times 10^{13}$  particles, even more

preferably no more than about  $1 \times 10^{12}$  particles, even more preferably no more than about  $1 \times 10^{11}$  particles, and most preferably no more than about  $1 \times 10^{10}$  particles (e.g., no more than about  $1 \times 10^9$  articles). Thus, the dose may contain a single dose of adenoviral vector with, for example, about  $1 \times 10^6$  particle units (pu), about  $2 \times 10^6$  pu, about  $4 \times 10^6$  pu, about  $1 \times 10^7$  pu, about  $2 \times 10^7$  pu, about  $4 \times 10^7$  pu, about  $1 \times 10^8$  pu, about  $2 \times 10^8$  pu, about  $4 \times 10^8$  pu, about  $1 \times 10^9$  pu, about  $2 \times 10^9$  pu, about  $4 \times 10^9$  pu, about  $1 \times 10^{10}$  pu, about  $2 \times 10^{10}$  pu, about  $4 \times 10^{10}$  pu, about  $1 \times 10^{11}$  pu, about  $2 \times 10^{11}$  pu, about  $4 \times 10^{11}$  pu, about  $1 \times 10^{12}$  pu, about  $2 \times 10^{12}$  pu, or about  $4 \times 10^{12}$  pu of adenoviral vector. See, for example, the adenoviral vectors in U.S. Patent No. 8,454,972 B2 to Nabel, et. al., granted on June 4, 2013; incorporated by reference herein, and the dosages at col 29, lines 36-58 thereof. In an embodiment herein, the adenovirus is delivered via multiple doses.

[0075] In an embodiment herein, the delivery is via an AAV. A therapeutically effective dosage for *in vivo* delivery of the AAV to a human is believed to be in the range of from about 20 to about 50 ml of saline solution containing from about  $1 \times 10^{10}$  to about  $1 \times 10^{50}$  functional AAV/ml solution. The dosage may be adjusted to balance the therapeutic benefit against any side effects. In an embodiment herein, the AAV dose is generally in the range of concentrations of from about  $1 \times 10^5$  to  $1 \times 10^{50}$  genomes AAV, from about  $1 \times 10^8$  to  $1 \times 10^{20}$  genomes AAV, from about  $1 \times 10^{10}$  to about  $1 \times 10^{16}$  genomes, or about  $1 \times 10^{11}$  to about  $1 \times 10^{16}$  genomes AAV. A human dosage may be about  $1 \times 10^{13}$  genomes AAV. Such concentrations may be delivered in from about 0.001 ml to about 100 ml, about 0.05 to about 50 ml, or about 10 to about 25 ml of a carrier solution. In a preferred embodiment, AAV is used with a titer of about  $2 \times 10^{13}$  viral genomes/milliliter, and each of the striatal hemispheres of a mouse receives one 500 nanoliter injection. Other effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves. See, for example, U.S. Patent No. 8,404,658 B2 to Hajjar, et al., granted on March 26, 2013, at col. 27, lines 45-60.

[0076] Lentiviral vectors have been disclosed as in the treatment for Parkinson's Disease, see, e.g., US Patent Publication No. 20120295960 and US Patent Nos. 7303910 and 7351585. Lentiviral vectors have also been disclosed for delivery to the Brain, see, e.g., US Patent Publication Nos. US20110293571; US20040013648, US20070025970, US20090111106 and US Patent No. US7259015. In another embodiment lentiviral vectors are used to deliver vectors to the brain of those being treated for a disease.

[0077] In an embodiment herein the delivery is via an lentivirus. Zou et al. administered about 10  $\mu$ l of a recombinant lentivirus having a titer of  $1 \times 10^9$  transducing units (TU)/ml by an intrathecal catheter. These sort of dosages can be adapted or extrapolated to use of a retroviral or lentiviral vector in the present invention. For transduction in tissues such as the brain, it is necessary to use very small volumes, so the viral preparation is concentrated by ultracentrifugation. The resulting preparation should have at least  $10^8$  TU/ml, preferably from  $10^8$  to  $10^9$  TU/ml, more preferably at least  $10^9$  TU/ml. Other methods of concentration such as ultrafiltration or binding to and elution from a matrix may be used.

[0078] In other embodiments the amount of lentivirus administered may be  $1 \times 10^5$  or about  $1 \times 10^5$  plaque forming units (PFU),  $5 \times 10^5$  or about  $5 \times 10^5$  PFU,  $1 \times 10^6$  or about  $1 \times 10^6$  PFU,  $5 \times 10^6$  or about  $5 \times 10^6$  PFU,  $1 \times 10^7$  or about  $1 \times 10^7$  PFU,  $5 \times 10^7$  or about  $5 \times 10^7$  PFU,  $1 \times 10^8$  or about  $1 \times 10^8$  PFU,  $5 \times 10^8$  or about  $5 \times 10^8$  PFU,  $1 \times 10^9$  or about  $1 \times 10^9$  PFU,  $5 \times 10^9$  or about  $5 \times 10^9$  PFU,  $1 \times 10^{10}$  or about  $1 \times 10^{10}$  PFU or  $5 \times 10^{10}$  or about  $5 \times 10^{10}$  PFU as total single dosage for an average human of 75 kg or adjusted for the weight and size and species of the subject. One of skill in the art can determine suitable dosage. Suitable dosages for a virus can be determined empirically.

[0079] In an embodiment herein the delivery is via a plasmid. In such plasmid compositions, the dosage should be a sufficient amount of plasmid to elicit a response. For instance, suitable quantities of plasmid DNA in plasmid compositions can be from about 0.1 to about 2 mg, from about 10  $\mu$ g to about 1 mg, from about 1  $\mu$ g to about 10  $\mu$ g from about 10 ng to about 1  $\mu$ g, or preferably from about 0.2  $\mu$ g to about 20  $\mu$ g.

[0080] Because the plasmid is the “vehicle” from which the protein is expressed, optimising vector design for maximal protein expression is essential (Lewis et al., (1999). *Advances in Virus Research* (Academic Press) 54: 129–88). Plasmids usually consist of a strong viral promoter to drive the *in vivo* transcription and translation of the gene (or cDNA) of interest (Mor, et al., (1995). *Journal of Immunology* 155 (4): 2039–2046). Promoters may be the SV40 promoter, Rous Sarcoma Virus (RSV) or the like. Intron A may sometimes be included to improve mRNA stability and hence increase protein expression (Leitner et al. (1997) *Journal of Immunology* 159 (12): 6112–6119). Plasmids also include a strong polyadenylation/transcriptional termination signal, such as bovine growth hormone or rabbit beta-globulin polyadenylation sequences (Alarcon et al., (1999). *Adv. Parasitol. Advances in*



Parasitology 42: 343–410; Robinson et al., (2000). *Adv. Virus Res. Advances in Virus Research* 55: 1–74; Böhmet et al., (1996). *Journal of Immunological Methods* 193 (1): 29–40).

[0081] DNA has been introduced into animal tissues by a number of different methods. The two most popular approaches are injection of DNA in saline, using a standard hypodermic needle, and gene gun delivery. A schematic outline of the construction of a DNA vaccine plasmid and its subsequent delivery by these two methods into a host is illustrated at *Scientific American* (Weiner et al., (1999) *Scientific American* 281 (1): 34–41).

[0082] Gene gun delivery ballistically accelerates plasmid DNA (pDNA) that has been adsorbed onto gold or tungsten microparticles into the target cells, using compressed helium as an accelerant (Alarcon et al., (1999). *Adv. Parasitol. Advances in Parasitology* 42: 343–410; Lewis et al., (1999). *Advances in Virus Research (Academic Press)* 54: 129–88).

[0083] Alternative delivery methods have included aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa, (Lewis et al., (1999). *Advances in Virus Research (Academic Press)* 54: 129–88) and topical administration of pDNA to the eye and vaginal mucosa (Lewis et al., (1999). *Advances in Virus Research (Academic Press)* 54: 129–88).

[0084] The method of delivery determines the dose of DNA required. Saline injections require variable amounts of DNA, from 10 µg-1 mg, whereas gene gun deliveries require 100 to 1000 times less DNA. Generally, 0.2 µg – 20 µg are required, although quantities as low as 16 ng have been reported. These quantities vary from species to species, with mice, for example, requiring approximately 10 times less DNA than primates. (See e.g., Sedegah et al., (1994). *Proceedings of the National Academy of Sciences of the United States of America* 91 (21): 9866–9870; Daheshiaet al., (1997). *The Journal of Immunology* 159 (4): 1945–1952; Chen et al., (1998). *The Journal of Immunology* 160 (5): 2425–2432; Sizemore (1995) *Science* 270 (5234): 299–302; Fynan et al., (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90 (24): 11478–82).

[0085] In another embodiment a nucleic acid that specifically represses the modulator can be used to treat a patient in need thereof. Nucleic acids that lead to repression may utilize RNAi based methods or CRISPR-Cas9 based systems.

[0086] Modulators of central nervous system diseases can be targeted for treatment using the CRISPR-Cas9 system. In one embodiment, the sequences in Table 9 can be used as guide sequences to target a CRISPR enzyme to the genes. Such a system can be used for gene editing

to knockout a gene or alter a mutated sequence. Additionally, CRISPR systems allow an increase in gene expression if fused to an activator of transcription. In an additional aspect of the invention, a Cas9 enzyme may comprise one or more mutations and may be used as a generic DNA binding protein with or without fusion to a functional domain. The mutations may be artificially introduced mutations or gain- or loss-of-function mutations. The mutations may include but are not limited to mutations in one of the catalytic domains (D10 and H840) in the RuvC and HNH catalytic domains, respectively. Further mutations have been characterized. In one aspect of the invention, the transcriptional activation domain may be VP64. In other aspects of the invention, the transcriptional repressor domain may be KRAB or SID4X. Other aspects of the invention relate to the mutated Cas 9 enzyme being fused to domains which include but are not limited to a transcriptional activator, repressor, a recombinase, a transposase, a histone remodeler, a demethylase, a DNA methyltransferase, a cryptochrome, a light inducible/controllable domain or a chemically inducible/controllable domain. In one embodiment, CRISPR is targeted to the Gpx6 gene. In another preferred embodiment, Gpx6 gene expression is increased.

[0087] In a further embodiment, the invention provides for methods to generate mutant tracrRNA and direct repeat sequences or mutant chimeric guide sequences that allow for enhancing performance of these RNAs in cells. Aspects of the invention also provide for selection of said sequences.

[0088] With respect to general information on CRISPR-Cas Systems, components thereof, and delivery of such components, including methods, materials, delivery vehicles, vectors, particles, AAV, and making and using thereof, including as to amounts and formulations, all useful in the practice of the instant invention, reference is made to: US Patents Nos. 8,999,641, 8,993,233, 8,945,839, 8,932,814, 8,906,616, 8,895,308, 8,889,418, 8,889,356, 8,871,445, 8,865,406, 8,795,965, 8,771,945 and 8,697,359; US Patent Publications US 2014-0310830 (US APP. Ser. No. 14/105,031), US 2014-0287938 A1 (U.S. App. Ser. No. 14/213,991), US 2014-0273234 A1 (U.S. App. Ser. No. 14/293,674), US2014-0273232 A1 (U.S. App. Ser. No. 14/290,575), US 2014-0273231 (U.S. App. Ser. No. 14/259,420), US 2014-0256046 A1 (U.S. App. Ser. No. 14/226,274), US 2014-0248702 A1 (U.S. App. Ser. No. 14/258,458), US 2014-0242700 A1 (U.S. App. Ser. No. 14/222,930), US 2014-0242699 A1 (U.S. App. Ser. No. 14/183,512), US 2014-0242664 A1 (U.S. App. Ser. No. 14/104,990), US 2014-0234972 A1

(U.S. App. Ser. No. 14/183,471), US 2014-0227787 A1 (U.S. App. Ser. No. 14/256,912), US 2014-0189896 A1 (U.S. App. Ser. No. 14/105,035), US 2014-0186958 (U.S. App. Ser. No. 14/105,017), US 2014-0186919 A1 (U.S. App. Ser. No. 14/104,977), US 2014-0186843 A1 (U.S. App. Ser. No. 14/104,900), US 2014-0179770 A1 (U.S. App. Ser. No. 14/104,837) and US 2014-0179006 A1 (U.S. App. Ser. No. 14/183,486), US 2014-0170753 (US App Ser No 14/183,429); European Patents EP 2 784 162 B1 and EP 2 771 468 B1; European Patent Applications EP 2 771 468 (EP13818570.7), EP 2 764 103 (EP13824232.6), and EP 2 784 162 (EP14170383.5); and PCT Patent Publications PCT Patent Publications WO 2014/093661 (PCT/US2013/074743), WO 2014/093694 (PCT/US2013/074790), WO 2014/093595 (PCT/US2013/074611), WO 2014/093718 (PCT/US2013/074825), WO 2014/093709 (PCT/US2013/074812), WO 2014/093622 (PCT/US2013/074667), WO 2014/093635 (PCT/US2013/074691), WO 2014/093655 (PCT/US2013/074736), WO 2014/093712 (PCT/US2013/074819), WO2014/093701 (PCT/US2013/074800), WO2014/018423 (PCT/US2013/051418), WO 2014/204723 (PCT/US2014/041790), WO 2014/204724 (PCT/US2014/041800), WO 2014/204725 (PCT/US2014/041803), WO 2014/204726 (PCT/US2014/041804), WO 2014/204727 (PCT/US2014/041806), WO 2014/204728 (PCT/US2014/041808), WO 2014/204729 (PCT/US2014/041809). Reference is also made to US provisional patent applications 61/758,468; 61/802,174; 61/806,375; 61/814,263; 61/819,803 and 61/828,130, filed on January 30, 2013; March 15, 2013; March 28, 2013; April 20, 2013; May 6, 2013 and May 28, 2013 respectively. Reference is also made to US provisional patent application 61/836,123, filed on June 17, 2013. Reference is additionally made to US provisional patent applications 61/835,931, 61/835,936, 61/836,127, 61/836, 101, 61/836,080 and 61/835,973, each filed June 17, 2013. Further reference is made to US provisional patent applications 61/862,468 and 61/862,355 filed on August 5, 2013; 61/871,301 filed on August 28, 2013; 61/960,777 filed on September 25, 2013 and 61/961,980 filed on October 28, 2013. Reference is yet further made to: PCT Patent applications Nos: PCT/US2014/041803, PCT/US2014/041800, PCT/US2014/041809, PCT/US2014/041804 and PCT/US2014/041806, each filed June 10, 2014 6/10/14; PCT/US2014/041808 filed June 11, 2014; and PCT/US2014/62558 filed October 28, 2014, and US Provisional Patent Applications Serial Nos.: 61/915,150, 61/915,301, 61/915,267 and 61/915,260, each filed December 12, 2013; 61/757,972 and 61/768,959, filed on January 29, 2013 and February 25, 2013; 61/835,936, 61/836,127,

61/836,101, 61/836,080, 61/835,973, and 61/835,931, filed June 17, 2013; 62/010,888 and 62/010,879, both filed June 11, 2014; 62/010,329 and 62/010,441, each filed June 10, 2014; 61/939,228 and 61/939,242, each filed February 12, 2014; 61/980,012, filed April 15, 2014; 62/038,358, filed August 17, 2014; 62/054,490, 62/055,484, 62/055,460 and 62/055,487, each filed September 25, 2014; and 62/069,243, filed October 27, 2014. Reference is also made to US provisional patent applications Nos. 62/055,484, 62/055,460, and 62/055,487, filed September 25, 2014; US provisional patent application 61/980,012, filed April 15, 2014; and US provisional patent application 61/939,242 filed February 12, 2014. Reference is made to PCT application designating, inter alia, the United States, application No. PCT/US14/41806, filed June 10, 2014. Reference is made to US provisional patent application 61/930,214 filed on January 22, 2014. Reference is made to US provisional patent applications 61/915,251; 61/915,260 and 61/915,267, each filed on December 12, 2013. Reference is made to US provisional patent application USSN 61/980,012 filed April 15, 2014. Reference is made to PCT application designating, inter alia, the United States, application No. PCT/US14/41806, filed June 10, 2014. Reference is made to US provisional patent application 61/930,214 filed on January 22, 2014. Reference is made to US provisional patent applications 61/915,251; 61/915,260 and 61/915,267, each filed on December 12, 2013.

[0089] Mention is also made of US application 62/091,455, filed, 12-Dec-14, PROTECTED GUIDE RNAS (PGRNAS); US application 62/096,708, 24-Dec-14, PROTECTED GUIDE RNAS (PGRNAS); US application 62/091,462, 12-Dec-14, DEAD GUIDES FOR CRISPR TRANSCRIPTION FACTORS; US application 62/096,324, 23-Dec-14, DEAD GUIDES FOR CRISPR TRANSCRIPTION FACTORS; US application 62/091,456, 12-Dec-14, ESCORTED AND FUNCTIONALIZED GUIDES FOR CRISPR-CAS SYSTEMS; US application 62/091,461, 12-Dec-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR GENOME EDITING AS TO HEMATOPOETIC STEM CELLS (HSCs); US application 62/094,903, 19-Dec-14, UNBIASED IDENTIFICATION OF DOUBLE-STRAND BREAKS AND GENOMIC REARRANGEMENT BY GENOME-WISE INSERT CAPTURE SEQUENCING; US application 62/096,761, 24-Dec-14, ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED ENZYME AND GUIDE SCAFFOLDS FOR SEQUENCE MANIPULATION; US application 62/098,059, 30-Dec-14, RNA-TARGETING SYSTEM; US application 62/096,656, 24-Dec-14, CRISPR HAVING OR

ASSOCIATED WITH DESTABILIZATION DOMAINS; US application 62/096,697, 24-Dec-14, CRISPR HAVING OR ASSOCIATED WITH AAV; US application 62/098,158, 30-Dec-14, ENGINEERED CRISPR COMPLEX INSERTIONAL TARGETING SYSTEMS; US application 62/151,052, 22-Apr-15, CELLULAR TARGETING FOR EXTRACELLULAR EXOSOMAL REPORTING; US application 62/054,490, 24-Sep-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR TARGETING DISORDERS AND DISEASES USING PARTICLE DELIVERY COMPONENTS; US application 62/055,484, 25-Sep-14, SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/087,537, 4-Dec-14, SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/054,651, 24-Sep-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR MODELING COMPETITION OF MULTIPLE CANCER MUTATIONS IN VIVO; US application 62/067,886, 23-Oct-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR MODELING COMPETITION OF MULTIPLE CANCER MUTATIONS IN VIVO; US application 62/054,675, 24-Sep-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS IN NEURONAL CELLS/TISSUES; US application 62/054,528, 24-Sep-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS IN IMMUNE DISEASES OR DISORDERS; US application 62/055,454, 25-Sep-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR TARGETING DISORDERS AND DISEASES USING CELL PENETRATION PEPTIDES (CPP); US application 62/055,460, 25-Sep-14, MULTIFUNCTIONAL-CRISPR COMPLEXES AND/OR OPTIMIZED ENZYME LINKED FUNCTIONAL-CRISPR COMPLEXES; US application 62/087,475, 4-Dec-14, FUNCTIONAL SCREENING WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/055,487, 25-Sep-14, FUNCTIONAL SCREENING WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/087,546, 4-Dec-14, MULTIFUNCTIONAL CRISPR COMPLEXES AND/OR OPTIMIZED ENZYME LINKED FUNCTIONAL-CRISPR COMPLEXES; and US

application 62/098,285, 30-Dec-14, CRISPR MEDIATED IN VIVO MODELING AND GENETIC SCREENING OF TUMOR GROWTH AND METASTASIS.

[0090] Each of these patents, patent publications, and applications, and all documents cited therein or during their prosecution (“appln cited documents”) and all documents cited or referenced in the appln cited documents, together with any instructions, descriptions, product specifications, and product sheets for any products mentioned therein or in any document therein and incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. All documents (e.g., these patents, patent publications and applications and the appln cited documents) are incorporated herein by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

[0091] Also with respect to general information on CRISPR-Cas Systems, mention is made of the following (also hereby incorporated herein by reference):

- Multiplex genome engineering using CRISPR/Cas systems. Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A., & Zhang, F. *Science* Feb 15;339(6121):819-23 (2013);
- RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Jiang W., Bikard D., Cox D., Zhang F, Marraffini LA. *Nat Biotechnol* Mar;31(3):233-9 (2013);
- One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. Wang H., Yang H., Shivalila CS., Dawlaty MM., Cheng AW., Zhang F., Jaenisch R. *Cell* May 9;153(4):910-8 (2013);
- Optical control of mammalian endogenous transcription and epigenetic states. Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. *Nature*. Aug 22;500(7463):472-6. doi: 10.1038/Nature12466. Epub 2013 Aug 23 (2013);
- Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. Ran, FA., Hsu, PD., Lin, CY., Gootenberg, JS., Konermann, S., Trevino, AE., Scott, DA., Inoue, A., Matoba, S., Zhang, Y., & Zhang, F. *Cell* Aug 28. pii: S0092-8674(13)01015-5 (2013-A);

- DNA targeting specificity of RNA-guided Cas9 nucleases. Hsu, P., Scott, D., Weinstein, J., Ran, FA., Konermann, S., Agarwala, V., Li, Y., Fine, E., Wu, X., Shalem, O., Cradick, TJ., Marraffini, LA., Bao, G., & Zhang, F. *Nat Biotechnol* doi:10.1038/nbt.2647 (2013);
- Genome engineering using the CRISPR-Cas9 system. Ran, FA., Hsu, PD., Wright, J., Agarwala, V., Scott, DA., Zhang, F. *Nature Protocols* Nov;8(11):2281-308 (2013-B);
- Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. Shalem, O., Sanjana, NE., Hartenian, E., Shi, X., Scott, DA., Mikkelsen, T., Heckl, D., Ebert, BL., Root, DE., Doench, JG., Zhang, F. *Science* Dec 12. (2013). [Epub ahead of print];
- Crystal structure of cas9 in complex with guide RNA and target DNA. Nishimasu, H., Ran, FA., Hsu, PD., Konermann, S., Shehata, SI., Dohmae, N., Ishitani, R., Zhang, F., Nureki, O. *Cell* Feb 27, 156(5):935-49 (2014);
- Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Wu X., Scott DA., Kriz AJ., Chiu AC., Hsu PD., Dadon DB., Cheng AW., Trevino AE., Konermann S., Chen S., Jaenisch R., Zhang F., Sharp PA. *Nat Biotechnol*. Apr 20. doi: 10.1038/nbt.2889 (2014);
- CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. *Cell* 159(2): 440-455 DOI: 10.1016/j.cell.2014.09.014(2014);
- Development and Applications of CRISPR-Cas9 for Genome Engineering, Hsu PD, Lander ES, Zhang F., *Cell*. Jun 5;157(6):1262-78 (2014).
- Genetic screens in human cells using the CRISPR/Cas9 system, Wang T, Wei JJ, Sabatini DM, Lander ES., *Science*. January 3; 343(6166): 80–84. doi:10.1126/science.1246981 (2014);
- Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation, Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE., (published online 3 September 2014) *Nat Biotechnol*. Dec;32(12):1262-7 (2014);

- *In vivo* interrogation of gene function in the mammalian brain using CRISPR-Cas9, Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, Sur M, Zhang F., (published online 19 October 2014) Nat Biotechnol. Jan;33(1):102-6 (2015);
- Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F., Nature. Jan 29;517(7536):583-8 (2015).
- A split-Cas9 architecture for inducible genome editing and transcription modulation, Zetsche B, Volz SE, Zhang F., (published online 02 February 2015) Nat Biotechnol. Feb;33(2):139-42 (2015);
- Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis, Chen S, Sanjana NE, Zheng K, Shalem O, Lee K, Shi X, Scott DA, Song J, Pan JQ, Weissleder R, Lee H, Zhang F, Sharp PA. Cell 160, 1246–1260, March 12, 2015 (multiplex screen in mouse), and
- *In vivo* genome editing using *Staphylococcus aureus* Cas9, Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F., (published online 01 April 2015), Nature. Apr 9;520(7546):186-91 (2015).
- Shalem et al., “High-throughput functional genomics using CRISPR-Cas9,” Nature Reviews Genetics 16, 299-311 (May 2015).
- Xu et al., “Sequence determinants of improved CRISPR sgRNA design,” Genome Research 25, 1147-1157 (August 2015).
- Parnas et al., “A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks,” Cell 162, 675-686 (July 30, 2015).
- Ramanan et al., CRISPR/Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus,” Scientific Reports 5:10833. doi: 10.1038/srep10833 (June 2, 2015)
- Nishimasu et al., Crystal Structure of *Staphylococcus aureus* Cas9,” Cell 162, 1113-1126 (Aug. 27, 2015)

each of which is incorporated herein by reference, may be considered in the practice of the instant invention, and discussed briefly below:



- Cong *et al.* engineered type II CRISPR-Cas systems for use in eukaryotic cells based on both *Streptococcus thermophilus* Cas9 and also *Streptococcus pyogenes* Cas9 and demonstrated that Cas9 nucleases can be directed by short RNAs to induce precise cleavage of DNA in human and mouse cells. Their study further showed that Cas9 as converted into a nicking enzyme can be used to facilitate homology-directed repair in eukaryotic cells with minimal mutagenic activity. Additionally, their study demonstrated that multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several at endogenous genomic loci sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology. This ability to use RNA to program sequence specific DNA cleavage in cells defined a new class of genome engineering tools. These studies further showed that other CRISPR loci are likely to be transplantable into mammalian cells and can also mediate mammalian genome cleavage. Importantly, it can be envisaged that several aspects of the CRISPR-Cas system can be further improved to increase its efficiency and versatility.
- Jiang *et al.* used the clustered, regularly interspaced, short palindromic repeats (CRISPR)–associated Cas9 endonuclease complexed with dual-RNAs to introduce precise mutations in the genomes of *Streptococcus pneumoniae* and *Escherichia coli*. The approach relied on dual-RNA:Cas9-directed cleavage at the targeted genomic site to kill unmutated cells and circumvents the need for selectable markers or counter-selection systems. The study reported reprogramming dual-RNA:Cas9 specificity by changing the sequence of short CRISPR RNA (crRNA) to make single- and multinucleotide changes carried on editing templates. The study showed that simultaneous use of two crRNAs enabled multiplex mutagenesis. Furthermore, when the approach was used in combination with recombineering, in *S. pneumoniae*, nearly 100% of cells that were recovered using the described approach contained the desired mutation, and in *E. coli*, 65% that were recovered contained the mutation.
- Wang *et al.* (2013) used the CRISPR/Cas system for the one-step generation of mice carrying mutations in multiple genes which were traditionally generated in multiple steps by sequential recombination in embryonic stem cells and/or time-consuming intercrossing of mice with a single mutation. The CRISPR/Cas system will greatly

accelerate the *in vivo* study of functionally redundant genes and of epistatic gene interactions.

- Konermann *et al.* (2013) addressed the need in the art for versatile and robust technologies that enable optical and chemical modulation of DNA-binding domains based CRISPR Cas9 enzyme and also Transcriptional Activator Like Effectors
- Ran *et al.* (2013-A) described an approach that combined a Cas9 nickase mutant with paired guide RNAs to introduce targeted double-strand breaks. This addresses the issue of the Cas9 nuclease from the microbial CRISPR-Cas system being targeted to specific genomic loci by a guide sequence, which can tolerate certain mismatches to the DNA target and thereby promote undesired off-target mutagenesis. Because individual nicks in the genome are repaired with high fidelity, simultaneous nicking *via* appropriately offset guide RNAs is required for double-stranded breaks and extends the number of specifically recognized bases for target cleavage. The authors demonstrated that using paired nicking can reduce off-target activity by 50- to 1,500-fold in cell lines and to facilitate gene knockout in mouse zygotes without sacrificing on-target cleavage efficiency. This versatile strategy enables a wide variety of genome editing applications that require high specificity.
- Hsu *et al.* (2013) characterized SpCas9 targeting specificity in human cells to inform the selection of target sites and avoid off-target effects. The study evaluated >700 guide RNA variants and SpCas9-induced indel mutation levels at >100 predicted genomic off-target loci in 293T and 293FT cells. The authors that SpCas9 tolerates mismatches between guide RNA and target DNA at different positions in a sequence-dependent manner, sensitive to the number, position and distribution of mismatches. The authors further showed that SpCas9-mediated cleavage is unaffected by DNA methylation and that the dosage of SpCas9 and sgRNA can be titrated to minimize off-target modification. Additionally, to facilitate mammalian genome engineering applications, the authors reported providing a web-based software tool to guide the selection and validation of target sequences as well as off-target analyses.
- Ran *et al.* (2013-B) described a set of tools for Cas9-mediated genome editing *via* non-homologous end joining (NHEJ) or homology-directed repair (HDR) in mammalian cells, as well as generation of modified cell lines for downstream functional studies. To

minimize off-target cleavage, the authors further described a double-nicking strategy using the Cas9 nickase mutant with paired guide RNAs. The protocol provided by the authors experimentally derived guidelines for the selection of target sites, evaluation of cleavage efficiency and analysis of off-target activity. The studies showed that beginning with target design, gene modifications can be achieved within as little as 1–2 weeks, and modified clonal cell lines can be derived within 2–3 weeks.

- Shalem *et al.* described a new way to interrogate gene function on a genome-wide scale. Their studies showed that delivery of a genome-scale CRISPR-Cas9 knockout (GeCKO) library targeted 18,080 genes with 64,751 unique guide sequences enabled both negative and positive selection screening in human cells. First, the authors showed use of the GeCKO library to identify genes essential for cell viability in cancer and pluripotent stem cells. Next, in a melanoma model, the authors screened for genes whose loss is involved in resistance to vemurafenib, a therapeutic that inhibits mutant protein kinase BRAF. Their studies showed that the highest-ranking candidates included previously validated genes NF1 and MED12 as well as novel hits NF2, CUL3, TADA2B, and TADA1. The authors observed a high level of consistency between independent guide RNAs targeting the same gene and a high rate of hit confirmation, and thus demonstrated the promise of genome-scale screening with Cas9.
- Nishimasu *et al.* reported the crystal structure of *Streptococcus pyogenes* Cas9 in complex with sgRNA and its target DNA at 2.5 Å resolution. The structure revealed a bilobed architecture composed of target recognition and nuclease lobes, accommodating the sgRNA:DNA heteroduplex in a positively charged groove at their interface. Whereas the recognition lobe is essential for binding sgRNA and DNA, the nuclease lobe contains the HNH and RuvC nuclease domains, which are properly positioned for cleavage of the complementary and non-complementary strands of the target DNA, respectively. The nuclease lobe also contains a carboxyl-terminal domain responsible for the interaction with the protospacer adjacent motif (PAM). This high-resolution structure and accompanying functional analyses have revealed the molecular mechanism of RNA-guided DNA targeting by Cas9, thus paving the way for the rational design of new, versatile genome-editing technologies.

- Wu *et al.* mapped genome-wide binding sites of a catalytically inactive Cas9 (dCas9) from *Streptococcus pyogenes* loaded with single guide RNAs (sgRNAs) in mouse embryonic stem cells (mESCs). The authors showed that each of the four sgRNAs tested targets dCas9 to between tens and thousands of genomic sites, frequently characterized by a 5-nucleotide seed region in the sgRNA and an NGG protospacer adjacent motif (PAM). Chromatin inaccessibility decreases dCas9 binding to other sites with matching seed sequences; thus 70% of off-target sites are associated with genes. The authors showed that targeted sequencing of 295 dCas9 binding sites in mESCs transfected with catalytically active Cas9 identified only one site mutated above background levels. The authors proposed a two-state model for Cas9 binding and cleavage, in which a seed match triggers binding but extensive pairing with target DNA is required for cleavage.
- Platt *et al.* established a Cre-dependent Cas9 knockin mouse. The authors demonstrated *in vivo* as well as *ex vivo* genome editing using adeno-associated virus (AAV)-, lentivirus-, or particle-mediated delivery of guide RNA in neurons, immune cells, and endothelial cells.
- Hsu *et al.* (2014) is a review article that discusses generally CRISPR-Cas9 history from yogurt to genome editing, including genetic screening of cells.
- Wang *et al.* (2014) relates to a pooled, loss-of-function genetic screening approach suitable for both positive and negative selection that uses a genome-scale lentiviral single guide RNA (sgRNA) library.
- Doench *et al.* created a pool of sgRNAs, tiling across all possible target sites of a panel of six endogenous mouse and three endogenous human genes and quantitatively assessed their ability to produce null alleles of their target gene by antibody staining and flow cytometry. The authors showed that optimization of the PAM improved activity and also provided an on-line tool for designing sgRNAs.
- Swiech *et al.* demonstrate that AAV-mediated SpCas9 genome editing can enable reverse genetic studies of gene function in the brain.
- Konermann *et al.* (2015) discusses the ability to attach multiple effector domains, e.g., transcriptional activator, functional and epigenomic regulators at appropriate positions on the guide such as stem or tetraloop with and without linkers.

- Zetsche *et al.* demonstrates that the Cas9 enzyme can be split into two and hence the assembly of Cas9 for activation can be controlled.
- Chen *et al.* relates to multiplex screening by demonstrating that a genome-wide *in vivo* CRISPR-Cas9 screen in mice reveals genes regulating lung metastasis.
- Ran *et al.* (2015) relates to SaCas9 and its ability to edit genomes and demonstrates that one cannot extrapolate from biochemical assays. Shalem *et al.* (2015) described ways in which catalytically inactive Cas9 (dCas9) fusions are used to synthetically repress (CRISPRi) or activate (CRISPRa) expression, showing advances using Cas9 for genome-scale screens, including arrayed and pooled screens, knockout approaches that inactivate genomic loci and strategies that modulate transcriptional activity.

End Edits

- Shalem *et al.* (2015) described ways in which catalytically inactive Cas9 (dCas9) fusions are used to synthetically repress (CRISPRi) or activate (CRISPRa) expression, showing advances using Cas9 for genome-scale screens, including arrayed and pooled screens, knockout approaches that inactivate genomic loci and strategies that modulate transcriptional activity.
- Xu *et al.* (2015) assessed the DNA sequence features that contribute to single guide RNA (sgRNA) efficiency in CRISPR-based screens. The authors explored efficiency of CRISPR/Cas9 knockout and nucleotide preference at the cleavage site. The authors also found that the sequence preference for CRISPRi/a is substantially different from that for CRISPR/Cas9 knockout.
- Parnas *et al.* (2015) introduced genome-wide pooled CRISPR-Cas9 libraries into dendritic cells (DCs) to identify genes that control the induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS). Known regulators of Tlr4 signaling and previously unknown candidates were identified and classified into three functional modules with distinct effects on the canonical responses to LPS.
- Ramanan *et al.* (2015) demonstrated cleavage of viral episomal DNA (cccDNA) in infected cells. The HBV genome exists in the nuclei of infected hepatocytes as a 3.2kb double-stranded episomal DNA species called covalently closed circular DNA (cccDNA), which is a key component in the HBV life cycle whose replication is not inhibited by current therapies. The authors showed that sgRNAs specifically targeting

highly conserved regions of HBV robustly suppresses viral replication and depleted cccDNA.

- Nishimasu *et al.* (2015) reported the crystal structures of SaCas9 in complex with a single guide RNA (sgRNA) and its double-stranded DNA targets, containing the 5'-TTGAAT-3' PAM and the 5'-TTGGGT-3' PAM. A structural comparison of SaCas9 with SpCas9 highlighted both structural conservation and divergence, explaining their distinct PAM specificities and orthologous sgRNA recognition.

[0092] Also, “Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing”, Shengdar Q. Tsai, Nicolas Wyvekens, Cyd Khayter, Jennifer A. Foden, Vishal Thapar, Deepak Reyon, Mathew J. Goodwin, Martin J. Aryee, J. Keith Joung *Nature Biotechnology* 32(6): 569-77 (2014), relates to dimeric RNA-guided FokI Nucleases that recognize extended sequences and can edit endogenous genes with high efficiencies in human cells.

[0093] Useful in the practice of the instant invention, reference is made to the article entitled BCL11A enhancer dissection by Cas9-mediated *in situ* saturating mutagenesis. Canver, M.C., Smith, E.C., Sher, F., Pinello, L., Sanjana, N.E., Shalem, O., Chen, D.D., Schupp, P.G., Vinjamur, D.S., Garcia, S.P., Luc, S., Kurita, R., Nakamura, Y., Fujiwara, Y., Maeda, T., Yuan, G., Zhang, F., Orkin, S.H., & Bauer, D.E. DOI:10.1038/nature15521, published online September 16, 2015, the article is herein incorporated by reference and discussed briefly below:

- Canver *et al.* involves novel pooled CRISPR-Cas9 guide RNA libraries to perform *in situ* saturating mutagenesis of the human and mouse BCL11A erythroid enhancers previously identified as an enhancer associated with fetal hemoglobin (HbF) level and whose mouse ortholog is necessary for erythroid BCL11A expression. This approach revealed critical minimal features and discrete vulnerabilities of these enhancers. Through editing of primary human progenitors and mouse transgenesis, the authors validated the BCL11A erythroid enhancer as a target for HbF reinduction. The authors generated a detailed enhancer map that informs therapeutic genome editing.

[0094] In addition, mention is made of PCT application PCT/US14/70057, Attorney Reference 47627.99.2060 and BI-2013/107 entitled “DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR TARGETING DISORDERS AND DISEASES USING PARTICLE DELIVERY

COMPONENTS (claiming priority from one or more or all of US provisional patent applications: 62/054,490, filed September 24, 2014; 62/010,441, filed June 10, 2014; and 61/915,118, 61/915,215 and 61/915,148, each filed on December 12, 2013) (“the Particle Delivery PCT”), incorporated herein by reference, with respect to a method of preparing an sgRNA-and-Cas9 protein containing particle comprising admixing a mixture comprising an sgRNA and Cas9 protein (and optionally HDR template) with a mixture comprising or consisting essentially of or consisting of surfactant, phospholipid, biodegradable polymer, lipoprotein and alcohol; and particles from such a process. For example, wherein Cas9 protein and sgRNA were mixed together at a suitable, e.g., 3:1 to 1:3 or 2:1 to 1:2 or 1:1 molar ratio, at a suitable temperature, e.g., 15-30C, e.g., 20-25C, e.g., room temperature, for a suitable time, e.g., 15-45, such as 30 minutes, advantageously in sterile, nuclease free buffer, e.g., 1X PBS. Separately, particle components such as or comprising: a surfactant, e.g., cationic lipid, e.g., 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP); phospholipid, e.g., dimyristoylphosphatidylcholine (DMPC); biodegradable polymer, such as an ethylene-glycol polymer or PEG, and a lipoprotein, such as a low-density lipoprotein, e.g., cholesterol were dissolved in an alcohol, advantageously a C<sub>1-6</sub> alkyl alcohol, such as methanol, ethanol, isopropanol, e.g., 100% ethanol. The two solutions were mixed together to form particles containing the Cas9-sgRNA complexes. Accordingly, sgRNA may be pre-complexed with the Cas9 protein, before formulating the entire complex in a particle. Formulations may be made with a different molar ratio of different components known to promote delivery of nucleic acids into cells (e.g. 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine (DMPC), polyethylene glycol (PEG), and cholesterol) For example DOTAP : DMPC : PEG : Cholesterol Molar Ratios may be DOTAP 100, DMPC 0, PEG 0, Cholesterol 0; or DOTAP 90, DMPC 0, PEG 10, Cholesterol 0; or DOTAP 90, DMPC 0, PEG 5, Cholesterol 5. DOTAP 100, DMPC 0, PEG 0, Cholesterol 0. That application accordingly comprehends admixing sgRNA, Cas9 protein and components that form a particle; as well as particles from such admixing. Aspects of the instant invention can involve particles; for example, particles using a process analogous to that of the Particle Delivery PCT, e.g., by admixing a mixture comprising sgRNA and/or Cas9 as in the instant invention and components that form a particle, e.g., as in the Particle Delivery PCT, to form a particle and particles from such admixing (or, of course, other particles involving sgRNA and/or Cas9 as in the instant invention).

[0095] In general, the CRISPR-Cas or CRISPR system is as used in the foregoing documents, such as WO 2014/093622 (PCT/US2013/074667) and refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated (“Cas”) genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a “direct repeat” and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a “spacer” in the context of an endogenous CRISPR system), or “RNA(s)” as that term is herein used (e.g., RNA(s) to guide Cas, such as Cas9, e.g. CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, “target sequence” refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, direct repeats may be identified *in silico* by searching for repetitive motifs that fulfill any or all of the following criteria: 1. found in a 2Kb window of genomic sequence flanking the type II CRISPR locus; 2. span from 20 to 50 bp; and 3. interspaced by 20 to 50 bp. In some embodiments, 2 of these criteria may be used, for instance 1 and 2, 2 and 3, or 1 and 3. In some embodiments, all 3 criteria may be used.

[0096] In embodiments of the invention the terms guide sequence and guide RNA, i.e. RNA capable of guiding Cas to a target genomic locus, are used interchangeably as in foregoing cited documents such as WO 2014/093622 (PCT/US2013/074667). In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable



algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies; available at [www.novocraft.com](http://www.novocraft.com)), ELAND (Illumina, San Diego, CA), SOAP (available at [soap.genomics.org.cn](http://soap.genomics.org.cn)), and Maq (available at [maq.sourceforge.net](http://maq.sourceforge.net)). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. Preferably the guide sequence is 10-30 nucleotides long. The ability of a guide sequence to direct sequence-specific binding of a CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.

[0097] In a classic CRISPR-Cas systems, the degree of complementarity between a guide sequence and its corresponding target sequence can be about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or 100%; a guide or RNA or sgRNA can be about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length; or guide or RNA or sgRNA can be less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length; and advantageously tracr RNA is 30 or 50 nucleotides in length. However, an aspect of the invention is to reduce off-target interactions, e.g., reduce the guide interacting with a target sequence having low complementarity. Indeed, in the examples, it is shown that the invention involves mutations that result in the CRISPR-Cas system being able to distinguish between target and off-target sequences that have greater than 80% to about 95% complementarity, e.g., 83%-84% or 88-89%

or 94-95% complementarity (for instance, distinguishing between a target having 18 nucleotides from an off-target of 18 nucleotides having 1, 2 or 3 mismatches). Accordingly, in the context of the present invention the degree of complementarity between a guide sequence and its corresponding target sequence is greater than 94.5% or 95% or 95.5% or 96% or 96.5% or 97% or 97.5% or 98% or 98.5% or 99% or 99.5% or 99.9%, or 100%. Off target is less than 100% or 99.9% or 99.5% or 99% or 99% or 98.5% or 98% or 97.5% or 97% or 96.5% or 96% or 95.5% or 95% or 94.5% or 94% or 93% or 92% or 91% or 90% or 89% or 88% or 87% or 86% or 85% or 84% or 83% or 82% or 81% or 80% complementarity between the sequence and the guide, with it advantageous that off target is 100% or 99.9% or 99.5% or 99% or 99% or 98.5% or 98% or 97.5% or 97% or 96.5% or 96% or 95.5% or 95% or 94.5% complementarity between the sequence and the guide.

[0098] In particularly preferred embodiments according to the invention, the guide RNA (capable of guiding Cas to a target locus) may comprise (1) a guide sequence capable of hybridizing to a genomic target locus in the eukaryotic cell; (2) a tracr sequence; and (3) a tracr mate sequence. All (1) to (3) may reside in a single RNA, i.e. an sgRNA (arranged in a 5' to 3' orientation), or the tracr RNA may be a different RNA than the RNA containing the guide and tracr sequence. The tracr hybridizes to the tracr mate sequence and directs the CRISPR/Cas complex to the target sequence.

[0099] The methods according to the invention as described herein comprehend inducing one or more mutations in a eukaryotic cell (in vitro, i.e. in an isolated eukaryotic cell) as herein discussed comprising delivering to cell a vector as herein discussed. The mutation(s) can include the introduction, deletion, or substitution of one or more nucleotides at each target sequence of cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations can include the introduction, deletion, or substitution of 1-75 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations can include the introduction, deletion, or substitution of 1, 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or 75 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations can include the introduction, deletion, or substitution of 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or 75 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations include the introduction, deletion, or substitution of 10, 11, 12, 13, 14, 15, 16, 17, 18,

19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or 75 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations can include the introduction, deletion, or substitution of 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or 75 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s). The mutations can include the introduction, deletion, or substitution of 40, 45, 50, 75, 100, 200, 300, 400 or 500 nucleotides at each target sequence of said cell(s) via the guide(s) RNA(s) or sgRNA(s).

**[00100]** For minimization of toxicity and off-target effect, it will be important to control the concentration of Cas mRNA and guide RNA delivered. Optimal concentrations of Cas mRNA and guide RNA can be determined by testing different concentrations in a cellular or non-human eukaryote animal model and using deep sequencing to analyze the extent of modification at potential off-target genomic loci. Alternatively, to minimize the level of toxicity and off-target effect, Cas nickase mRNA (for example *S. pyogenes* Cas9 with the D10A mutation) can be delivered with a pair of guide RNAs targeting a site of interest. Guide sequences and strategies to minimize toxicity and off-target effects can be as in WO 2014/093622 (PCT/US2013/074667); or, via mutation as herein.

**[00101]** Typically, in the context of an endogenous CRISPR system, formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of a CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence.

**[00102]** The nucleic acid molecule encoding a Cas is advantageously codon optimized Cas. An example of a codon optimized sequence, is in this instance a sequence optimized for expression in a eukaryote, e.g., humans (i.e. being optimized for expression in humans), or for another eukaryote, animal or mammal as herein discussed; see, e.g., SaCas9 human codon optimized sequence in WO 2014/093622 (PCT/US2013/074667). Whilst this is preferred, it will be appreciated that other examples are possible and codon optimization for a host species other

than human, or for codon optimization for specific organs is known. In some embodiments, an enzyme coding sequence encoding a Cas is codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, or non-human eukaryote or animal or mammal as herein discussed, e.g., mouse, rat, rabbit, dog, livestock, or non-human mammal or primate. In some embodiments, processes for modifying the germ line genetic identity of human beings and/or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes, may be excluded. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon (e.g. about or more than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at [www.kazusa.or.jp/codon/](http://www.kazusa.or.jp/codon/) and these tables can be adapted in a number of ways. See Nakamura, Y., et al. "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" *Nucl. Acids Res.* 28:292 (2000). Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge (Aptagen; Jacobus, PA), are also available. In some embodiments, one or more codons (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more, or all codons) in a sequence encoding a Cas correspond to the most frequently used codon for a particular amino acid.

[00103] In certain embodiments, the methods as described herein may comprise providing a Cas transgenic cell in which one or more nucleic acids encoding one or more guide RNAs are provided or introduced operably connected in the cell with a regulatory element comprising a

promoter of one or more gene of interest. As used herein, the term “Cas transgenic cell” refers to a cell, such as a eukaryotic cell, in which a Cas gene has been genomically integrated. The nature, type, or origin of the cell are not particularly limiting according to the present invention. Also the way how the Cas transgene is introduced in the cell is may vary and can be any method as is known in the art. In certain embodiments, the Cas transgenic cell is obtained by introducing the Cas transgene in an isolated cell. In certain other embodiments, the Cas transgenic cell is obtained by isolating cells from a Cas transgenic organism. By means of example, and without limitation, the Cas transgenic cell as referred to herein may be derived from a Cas transgenic eukaryote, such as a Cas knock-in eukaryote. Reference is made to WO 2014/093622 (PCT/US13/74667), incorporated herein by reference. Methods of US Patent Publication Nos. 20120017290 and 20110265198 assigned to Sangamo BioSciences, Inc. directed to targeting the Rosa locus may be modified to utilize the CRISPR Cas system of the present invention. Methods of US Patent Publication No. 20130236946 assigned to Collectis directed to targeting the Rosa locus may also be modified to utilize the CRISPR Cas system of the present invention. By means of further example reference is made to Platt et. al. (Cell; 159(2):440-455 (2014)), describing a Cas9 knock-in mouse, which is incorporated herein by reference. The Cas transgene can further comprise a Lox-Stop-polyA-Lox(LSL) cassette thereby rendering Cas expression inducible by Cre recombinase. Alternatively, the Cas transgenic cell may be obtained by introducing the Cas transgene in an isolated cell. Delivery systems for transgenes are well known in the art. By means of example, the Cas transgene may be delivered in for instance eukaryotic cell by means of vector (e.g., AAV, adenovirus, lentivirus) and/or particle and/or nanoparticle delivery, as also described herein elsewhere.

**[00104]** It will be understood by the skilled person that the cell, such as the Cas transgenic cell, as referred to herein may comprise further genomic alterations besides having an integrated Cas gene or the mutations arising from the sequence specific action of Cas when complexed with RNA capable of guiding Cas to a target locus, such as for instance one or more oncogenic mutations, as for instance and without limitation described in Platt et al. (2014), Chen et al., (2014) or Kumar et al.. (2009).

**[00105]** In some embodiments, the Cas sequence is fused to one or more nuclear localization sequences (NLSs), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the Cas comprises about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or

more NLSs at or near the amino-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the carboxy-terminus, or a combination of these (e.g. zero or at least one or more NLS at the amino-terminus and zero or at one or more NLS at the carboxy terminus). When more than one NLS is present, each may be selected independently of the others, such that a single NLS may be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. In a preferred embodiment of the invention, the Cas comprises at most 6 NLSs. In some embodiments, an NLS is considered near the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus. Non-limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO: X); the NLS from nucleoplasmin (e.g. the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK) (SEQ ID NO: X); the c-myc NLS having the amino acid sequence PAAKRVKLD (SEQ ID NO: X) or RQRRNELKRSP (SEQ ID NO: X); the hRNPA1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: X); the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: X) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO: X) and PPKKARED (SEQ ID NO: X) of the myoma T protein; the sequence POPKKKPL (SEQ ID NO: X) of human p53; the sequence SALIKKKKKMAP (SEQ ID NO: X) of mouse c-abl IV; the sequences DRLRR (SEQ ID NO: X) and PKQKKRK (SEQ ID NO: X) of the influenza virus NS1; the sequence RKLKKKIKKL (SEQ ID NO: X) of the Hepatitis virus delta antigen; the sequence REKKKFLKRR (SEQ ID NO: X) of the mouse Mx1 protein; the sequence KRKGDEVDGVDEVAKKKS (SEQ ID NO: X) of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: X) of the steroid hormone receptors (human) glucocorticoid. In general, the one or more NLSs are of sufficient strength to drive accumulation of the Cas in a detectable amount in the nucleus of a eukaryotic cell. In general, strength of nuclear localization activity may derive from the number of NLSs in the Cas, the particular NLS(s) used, or a combination of these factors. Detection of accumulation in the nucleus may be performed by any suitable technique. For example, a detectable marker may be fused to the Cas, such that location within a cell may be visualized, such as in combination with a means for detecting the location of the nucleus (e.g. a stain specific for the

nucleus such as DAPI). Cell nuclei may also be isolated from cells, the contents of which may then be analyzed by any suitable process for detecting protein, such as immunohistochemistry, Western blot, or enzyme activity assay. Accumulation in the nucleus may also be determined indirectly, such as by an assay for the effect of CRISPR complex formation (e.g. assay for DNA cleavage or mutation at the target sequence, or assay for altered gene expression activity affected by CRISPR complex formation and/or Cas enzyme activity), as compared to a control not exposed to the Cas or complex, or exposed to a Cas lacking the one or more NLSs.

[00106] In certain aspects the invention involves vectors, e.g. for delivering or introducing in a cell Cas and/or RNA capable of guiding Cas to a target locus (i.e. guide RNA), but also for propagating these components (e.g. in prokaryotic cells). As used herein, a “vector” is a tool that allows or facilitates the transfer of an entity from one environment to another. It is a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. Generally, a vector is capable of replication when associated with the proper control elements. In general, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses (AAVs)). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-

linked. Such vectors are referred to herein as “expression vectors.” Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[00107] Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g. in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). With regards to recombination and cloning methods, mention is made of U.S. patent application 10/815,730, published September 2, 2004 as US 2004-0171156 A1, the contents of which are herein incorporated by reference in their entirety.

[00108] The vector(s) can include the regulatory element(s), e.g., promoter(s). The vector(s) can comprise Cas encoding sequences, and/or a single, but possibly also can comprise at least 3 or 8 or 16 or 32 or 48 or 50 guide RNA(s) (e.g., sgRNAs) encoding sequences, such as 1-2, 1-3, 1-4 1-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-8, 3-16, 3-30, 3-32, 3-48, 3-50 RNA(s) (e.g., sgRNAs). In a single vector there can be a promoter for each RNA (e.g., sgRNA), advantageously when there are up to about 16 RNA(s) (e.g., sgRNAs); and, when a single vector provides for more than 16 RNA(s) (e.g., sgRNAs), one or more promoter(s) can drive expression of more than one of the RNA(s) (e.g., sgRNAs), e.g., when there are 32 RNA(s) (e.g., sgRNAs), each promoter can drive expression of two RNA(s) (e.g., sgRNAs), and when there are 48 RNA(s) (e.g., sgRNAs), each promoter can drive expression of three RNA(s) (e.g., sgRNAs). By simple arithmetic and well established cloning protocols and the teachings in this disclosure one skilled in the art can readily practice the invention as to the RNA(s) (e.g., sgRNA(s) for a suitable exemplary vector such as AAV, and a suitable promoter such as the U6 promoter, e.g., U6-sgRNAs. For example, the packaging limit of AAV is ~4.7 kb. The length of a single U6-sgRNA (plus restriction sites for cloning) is 361 bp. Therefore, the skilled person can readily fit about 12-16, e.g., 13 U6-sgRNA cassettes in a single vector. This can be assembled by any suitable means, such as a golden gate strategy used for TALE assembly ([www.genome-engineering.org/taleffectors/](http://www.genome-engineering.org/taleffectors/)). The skilled person can also use a tandem guide strategy to increase the number of U6-sgRNAs by



approximately 1.5 times, e.g., to increase from 12-16, e.g., 13 to approximately 18-24, e.g., about 19 U6-sgRNAs. Therefore, one skilled in the art can readily reach approximately 18-24, e.g., about 19 promoter-RNAs, e.g., U6-sgRNAs in a single vector, e.g., an AAV vector. A further means for increasing the number of promoters and RNAs, e.g., sgRNA(s) in a vector is to use a single promoter (e.g., U6) to express an array of RNAs, e.g., sgRNAs separated by cleavable sequences. And an even further means for increasing the number of promoter-RNAs, e.g., sgRNAs in a vector, is to express an array of promoter-RNAs, e.g., sgRNAs separated by cleavable sequences in the intron of a coding sequence or gene; and, in this instance it is advantageous to use a polymerase II promoter, which can have increased expression and enable the transcription of long RNA in a tissue specific manner. (see, e.g., [nar.oxfordjournals.org/content/34/7/e53.short](http://nar.oxfordjournals.org/content/34/7/e53.short), [www.nature.com/mt/journal/v16/n9/abs/mt2008144a.html](http://www.nature.com/mt/journal/v16/n9/abs/mt2008144a.html)). In an advantageous embodiment, AAV may package U6 tandem sgRNA targeting up to about 50 genes. Accordingly, from the knowledge in the art and the teachings in this disclosure the skilled person can readily make and use vector(s), e.g., a single vector, expressing multiple RNAs or guides or sgRNAs under the control or operatively or functionally linked to one or more promoters—especially as to the numbers of RNAs or guides or sgRNAs discussed herein, without any undue experimentation.

[00109] The guide RNA(s), e.g., sgRNA(s) encoding sequences and/or Cas encoding sequences, can be functionally or operatively linked to regulatory element(s) and hence the regulatory element(s) drive expression. The promoter(s) can be constitutive promoter(s) and/or conditional promoter(s) and/or inducible promoter(s) and/or tissue specific promoter(s). The promoter can be selected from the group consisting of RNA polymerases, pol I, pol II, pol III, T7, U6, H1, retroviral Rous sarcoma virus (RSV) LTR promoter, the cytomegalovirus (CMV) promoter, the SV40 promoter, the dihydrofolate reductase promoter, the  $\beta$ -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 $\alpha$  promoter. An advantageous promoter is the promoter is U6.

[00110] Mice used in experiments are about 20g. From that which is administered to a 20g mouse, one can extrapolate to scale up dosing to a 70kg individual. In another preferred embodiment the doses herein are scaled up based on an average 70 kg individual to treat a

patient in need thereof. The frequency of administration is within the ambit of the medical or veterinary practitioner (e.g., physician, veterinarian), or scientist skilled in the art.

[00111] In other embodiments, any of the proteins, antagonists, antibodies, agonists, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described herein. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects. In a preferred embodiment, Huntington's Disease is treated by use of an identified modulator, as described herein, in conjunction with a known treatment. Treating with a modulator by either effecting its expression or by overexpressing the protein may not completely alleviate symptoms. Therefore, other drugs that specifically target the symptoms can be combined with that of a modulator. Central nervous system diseases are associated with oxidative stress as well as having neurological symptoms that lead to both mental and physical abnormalities. A combination therapy may be used to synergistically alleviate these symptoms. Antioxidants and Gpx mimetics may be used in combination with other known treatments when a modulator involved in oxidative stress is identified. The antioxidant ebselen may be used at about 300 mg per day. Such treatments may comprise Tetrabenazine, neuroleptics, benzodiazepines, amantadine, anti Parkinson's drugs and valproic acid. Tetrabenazine is used to treat Huntington's chorea (uncontrolled muscle movements) and can be given in doses of 12.5 mg orally weekly to a maximum dose of 37.5 to 50 mg daily. Preferably less than 25 mg is administered. In combination, the dosage may be less than 12.5 mg. Neuroleptics are used to treat psychotic disorders and may be given in a dose of 10 to 200 mg daily. Benzodiazepines are used as sedatives, hypnotics, anxiolytics, anticonvulsants and muscle relaxants. They may be administered in doses of between 3 to 6 mg/day. Amantadine is an antiviral medication and may be used in doses of 200 mg/day, up to 400 mg per day. Valproic acid is used to treat various types of seizure disorders and can be administered in doses of 5 to 60 mg/kg per day in divided doses. In one embodiment of the invention, the medicament may further comprise but is not limited to the following Parkinson's drugs: levodopa, dopamine agonists, catechol O-

methyltransferase (COMT) inhibitors, monoamine oxidase B (MAO B) inhibitors, anticholinergic agents, or a combination thereof.

[00112] In another embodiment, antibodies are developed that bind specifically to the modulators using known methods in the art. In one embodiment the antibodies are polyclonal. In another embodiment the antibodies are monoclonal. In one embodiment the antibodies are generated against the full length protein. In another embodiment the antibodies are generated against antigenic fragments of the modulators. In one embodiment the antibodies are produced in sheep. In one embodiment the antibodies are produced in rabbits. In one embodiment the antibodies are produced in mice. In one embodiment the antibodies are produced in goats. In one embodiment the antibodies are used to study central nervous system diseases by staining tissue samples. In one embodiment the antibodies are used to determine protein quantity.

[00113] In another embodiment, modulators of central nervous system diseases can be used for diagnostic or prognostic screening. In one embodiment a modulator found to be synthetically lethal when knocked down in the screening method, would be a positive prognostic marker of disease outcome. In a preferred embodiment the modulator is Gpx6. In one embodiment a modulator found to be synthetically lethal when overexpressed in the screening method, would be a negative prognostic marker of disease outcome. In a preferred embodiment the protein expression of the modulator is determined. This may be performed with antibodies in western blots or in tissue staining. In another preferred embodiment gene expression is determined. This may be performed using microarrays, RT-PCR, quantitative PCR, or northern blot.

[00114] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R.I. Freshney, ed. (1987)).

[00115] The practice of the present invention employs, unless otherwise indicated, conventional techniques for generation of genetically modified mice. See Marten H. Hofker and Jan van Deursen, TRANSGENIC MOUSE METHODS AND PROTOCOLS, 2nd edition (2011).

[00116] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[00117] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

## **Examples**

### *Example 1*

#### **Differential Gene Expression Profiling and Pathways Analysis**

[00118] This example describes cell-type specific molecular profiles of cell populations during normal mouse brain aging and normal age-associated molecular pathways in various neurodegenerative disease-relevant cell types (Figure 1 and Tables 1-8). Applicants employed the translating ribosome affinity purification (TRAP) methodology (Heiman et al., (2008) Cell 135(4):738-748; Doyle et al., (2008) Cell 135(4):749-762) to create cell-type specific molecular profiles of cell populations during normal mouse brain aging. Mice aged 6 weeks or 2 years and 6 weeks from the *Drd1::EGFP-L10a* or *Drd2::EGFP-L10a* Bacterial Artificial Chromosome (BAC) transgenic lines (n=4 each group) were decapitated and brain tissue was immediately dissected and used for TRAP RNA purifications as previously described (Heiman et al., (2008)). RNA was used to interrogate Affymetrix Mouse Exon Chips (Affymetrix, Santa Clara, CA) after amplification using the NuGEN Ovation protocol for probe preparation (NuGEN, San Carlos, CA). Genes differentially expressed across aging were identified as previously described (Heiman et al., (2008), Heiman et al., (2014) Nat Protoc. 2014;9(6):1282-91) using Welch's t-test. Applicants defined significantly differentially expressed genes as those having any probe-sets with  $\geq 1.2$ -fold change and a Benjamini-Hochberg adjusted p-value from Welch's t test of  $\leq 0.05$ . For each comparison group, the set of statistically significant differentially expressed genes, independent of magnitude of change, was compared against the Wikipathways gene sets to compute overlaps. Statistical significance of gene set overlaps was assessed by a hypergeometric test.

[00119] **Results.** Each cell type displayed a unique pattern of gene expression changes that was associated with aging (Tables 1-4 and Figure 1). Only 5 genes, including 2 pseudogenes, displayed altered expression with aging in all cell types (Tnnt2, Gm5425, Rnd3, Pisd, and Pisd-ps3), indicating that there is not a general aging program across these cell types studied, but rather that even closely related cell types show distinct molecular changes during normal aging.

[00120] Pathways analysis of genes whose expression was altered revealed several molecular pathways altered with aging in each cell type (Tables 5-8) In Drd2-expressing striatal neurons, which displayed the most number of altered gene pathways during aging, “glutathione-mediated detoxification” and “glutathione redox reactions” were amongst the top gene pathways altered with age (including the genes Gsta3, Gsta4, Gstm1, Gstm6, Gpx1, Gpx2, and Gpx6). Oxidative damage has long been linked to aging (Harman et al., 1956). Given that oxidative damage to DNA, proteins, and lipids have all been reported to increase with age in the brain (Mecocci et al., (1993) *Annals of neurology* 34(4):609-616; Dei, Takeda, et al., (2002) *Acta neuropathologica* 104(2):113-122; Smith, Carney et al., (1991) *Proceedings of the National Academy of Sciences of the USA* 88(23):10540-10543), the increases to glutathione-dependent enzymes reported here likely reflect a homeostatic neuronal response to increased oxidative damage in this cell population.

### *Example 2*

#### **Synthetic Lethal Knockdown Screen For Genes Enhancing Huntingtin Toxicity**

[00121] This example describes results of the SLIC genetic screening platform used in the mammalian nervous system. The SLIC screening platform utilizes individual neurons in a brain region as a genetic screening vehicle, as opposed to one mouse being used as a screening vehicle (Figure 2). Specifically, genes were screened for synthetic lethality in a Huntington’s disease mouse model that, when knocked down, would enhance mutant huntingtin toxicity. R6/2 mice (Mangiarini et al., (1996) *Cell* 87(3):493-506) or control littermates 6 weeks of age were anesthetized with a mixture of ketamine (Putney Inc., Portland, ME) and xylazine (Lloyd Inc., Shenandoah, IA) and mounted on a Leica (Solms, Germany) mouse stereotaxic frame in a flat-skull position. Viral pools of lentiviruses carrying barcoded short hairpin RNAs (shRNAs) were injected bilaterally into mouse striata of disease and control littermates. One microliter of the barcoded lentiviral pools was injected at each of the following four coordinates (in mm relative

to bregma, sagittal suture and dural surface): AP=0.3, L=2, DV=-3.7 ; AP=0.3, L=-2, DV=-3.7; AP=0.9, L=1.7, DV=-3.3; AP=0.9, L=-1.7, DV=-3.3. The lentiviruses carrying barcoded short hairpin RNAs (shRNAs) included 96 shRNA elements for the screen (Table 9), which included a positive control shRNA, negative control shRNAs, and experimental shRNAs that targeted 24 genes, with an average of 3.4 hairpins per gene. The 24 target genes were selected due to their high magnitude change in the aging TRAP study described in example 1 or else a previously reported link to Huntington's disease.

[00122] Two days, four weeks, or six weeks after lentiviral injections, mice were sacrificed and brain tissue was processed for genomic DNA extraction using a Qiagen kit (Qiagen, Hilden, Germany). Illumina sequencing and deconvolution were performed as previously described to determine lentiviral barcode representation (Ashton, Jordan, et al., 2012). (See also: <http://www.broadinstitute.org/rnai/public/resources/protocols>). Significance of screen results was calculated with the RIGER software as previously described (Luo, Cheung, Subramanian, et al. (2008). (See also: <http://www.broadinstitute.org/cancer/software/GENE-E/>).

[00123] **Results.** Based on test injections, Applicants calculate that up to  $2.8 \times 10^5$  striatal cells are targeted per mouse (Figure 3), and that over 80% of viral-transduced cells are neurons (Figure 4). Comparison of viral barcode representation in the wild-type control (non-model) mouse striatal samples at 4 weeks versus 2 days revealed that the positive control lentivirus, carrying an shRNA targeting the Psm2 gene product (a proteasomal subunit, depletion of which is expected to lead to cell death), was greatly reduced in representation, while negative controls, which have no expected target in the mouse genome, were not reduced in representation (Figure 5A). ShRNAs that led to enhanced cell death in R6/2 mice and not control mice revealed genes that display synthetic lethality with mutant huntingtin. Comparison of the R6/2 Huntington's disease model mice versus control littermates at the 4 and 6 weeks experimental time-points revealed that all shRNAs targeting Gpx6, a glutathione peroxidase that by homology is predicted to detoxify H<sub>2</sub>O<sub>2</sub> to water, demonstrated synthetic lethality with mutant huntingtin (p value = 0.0036 at 4 weeks of incubation; p value = 0.0321 at 6 weeks of incubation) (Figure 5B and 5C and Tables 10, 11, and 12). No other targeted gene displayed statistically significant synthetic lethality at either screening time-point. Importantly, other shRNAs that affected general health of cells did not exhibit synthetic lethality with mutant huntingtin, and were lost approximately equally in both R6/2 mouse brain and controls (Figure 5B).

*Example 3***Gpx6 Function and Expression**

[00124] This example describes Gpx6 function and expression. Applicants assessed Gpx6 distribution across brain region and age. Gpx6, high-titer adeno-associated virus serotype 9 (AAV9) was used to overexpress FLAG-tagged Gpx6 or the TRAP construct (control) in the striatum of the R6/2 model and control mice by bilateral injection at the following coordinates: AP=0.6, L=1.85, DV=-3.5; and AP=0.6, L=-1.85, DV=-3.5. AAV was used with a titer of about  $2 \times 10^{13}$  viral genomes/milliliter, and each of the striatal hemispheres received one 500 nanoliter injection in the Gpx6 over expression study. Virus vehicle was either phosphate-buffered saline or Hank's Balanced Salt Solution. Mice were 6 weeks of age upon injection with the AAV9 construct, and were tested in an open field assay at two weeks post injection. In a separate series of experiments, mice were also injected with AAV9, at the same coordinates, but with one striatal hemisphere receiving the FLAG-tagged Gpx6 AAV9 and one striatal hemisphere receiving the TRAP construct (control) AAV9. These mice were perfused for indirect immunofluorescent staining at two weeks post injection.

[00125] **Results.** Applicants found that Gpx6 expression is down-regulated in the brains of Huntington's disease model mice (Figure 6). Applicants also found Gpx6 to be highly expressed in the olfactory bulb, striatum, and frontal cerebral cortex (Figure 7) and, confirming the TRAP results in example 1, observed that Gpx6 expression increases with age (Figure 8). Over-expression of Gpx6 showed a therapeutic effect on phenotype progression in a Huntington's disease mouse model. Two weeks after viral injection, Applicants observed a dramatic rescue of open-field motor behavior in R6/2 mice, but no effect of viral transduction on motor behavior in wild-type mice (Figure 9A). Finally, analysis of a molecular marker of Huntington's disease progression, loss of DARPP-32 striatal expression (Bibb et al., (2000) Proceedings of the National Academy of Sciences of the USA 97(12):6809-6814), revealed that Gpx6 over-expression also increases DARPP-32 expression in the R6/2 model (Figure 9B).

*Example 4***Effects of Gpx6 Overexpression on Parkinson's Disease Model Phenotype Progression**

[00126] This example describes a decrease in phenotype progression in a Parkinson's disease mouse model after overexpression of Gpx6. Based on the ability of Gpx6 overexpression to

delay the emergence of several Huntington's disease phenotypes in mouse models of the disease, Applicant's tested the effects of Gpx6 overexpression on a mouse model of Parkinson's disease (PD). The PD model overexpresses human alpha-synuclein that contains two PD-associated mutations, A30P and A53T (The Jackson Laboratories stock # 008239). Starting at 2-3 months of age, these PD model mice are hyperactive, but then start to show a reduction in activity at approximately 16 months of age. In order to test the effect of Gpx6 overexpression on the disease course in this mouse model, Applicant's injected mice at 6 weeks of age with a control (TRAP construct) or Gpx6 overexpression virus, allowed the mice to recover, and aged them to a time-point where it would be expected to see a behavioral phenotype. The data shows that Gpx6 overexpression has a therapeutic benefit in this mouse model of PD, as Gpx6 overexpression reduced the hyperactivity seen at this age in this PD model (Figure 10).

#### *Methods*

[00127] **Animal Usage.** All animal experiments were conducted with the approval of the Massachusetts Institute of Technology Animal Care and Use Committee. Mice were housed with food and water provided *ad libitum*. Experiments were conducted with *Drd1::EGFP-L10a* or *Drd2::EGFP-L10a* Bacterial Artificial Chromosome (BAC) transgenic (Heiman et al., 2008), adult (6 weeks old and 2 years, 6 weeks old) female mice on the C57BL/6J strain background, or with R6/2 model mice (Mangiarini et al., 1996) (B6CBA-Tg(HDexon1)62Gpb/1J, Jackson Laboratory stock #002810) at 5-12 weeks of age.

[00128] **In vitro Validation of Lentiviral Knockdown Efficiency.** HEK293T/17 cells (ATCC, Manassas, VA) were grown in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA) and transfected with FLAG-tagged Gpx6 over-expression constructs (Origene, Rockville, MD) using the FuGENE6HD reagent (Promega, Madison WI) following the manufacturer's instructions. One day after transfection, cells were transduced with Gpx6-targeting shRNA lentiviruses, and cell lysates were prepared for standard Western blotting two days later by lysing cells directly in Western blot sample buffer.

[00129] **Indirect Immunofluorescent Staining.** Mouse brain tissue was prepared and stained as previously described (Heiman et al., 2008), using the following primary antibodies: DARPP-32 (Cell Signaling Technology, Beverly, MA, antibody19A3, 1:1,000 dilution), GFP (Abcam,



Cambridge, England, antibody ab6556, 1:5,000 dilution), NeuN (1:100 dilution), and GFAP (1:1,000 dilution).

**[00130] Lentiviral Library Preparation.** Lentivirus was prepared and pooled as previously described (Root, Sabatini, et al., 2006). Lentiviruses were concentrated by centrifugation at 20,000 x g through a 20% sucrose cushion in a SW32Ti rotor (Beckman Coulter, Inc., Pasadena, CA), using an Optima L-90K centrifuge (Beckman Coulter, Inc., Pasadena, CA), and resuspended in Hank's Balanced Salt Solution (HBSS) to an approximate titer of  $5 \times 10^5$  functional particles/ $\mu$ l before stereotaxic injection.

**[00131] Open Field Behavioral Testing.** Mice were placed in a non-illuminated open field platform (19 in length x 20 in width x 15 in high; with 16 infrared beams each in the X and Y axis) housed within an environmental control chamber (both from Omnitech Electronic, Inc., Columbus, OH) during the first half of their light phase. Activity measurements captured by infrared beam breaks were collected in 10 min intervals, for a total of 60 min.

**[00132] Quantitative PCR.** RNA was purified from aged and control mouse brain tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). Complementary cDNA was produced using the SuperScript III kit (Invitrogen, Carlsbad, CA). Alternatively, to profile gene expression across brain regions, a commercially available mouse brain cDNA panel was used (Zyagen, San Diego, CA). Quantitative PCR was performed with 100 ng of cDNA, Taqman reagents and primers (Invitrogen, Carlsbad, CA), and a LightCycler480 (Roche, Basel Switzerland). Taqman primers used were as follows:

TaqMan Gene Expression Assay ID: Mm00607939\_s1, Gene Symbol: Actb, mCG23209

TaqMan Gene Expression Assay ID: Mm00513979\_m1, Gene Symbol: Gpx6

**[00133] Generation of a Gpx6 Polyclonal Antibody.** As no commercial antibody that is specific for Gpx6 is available, Applicant's developed a rabbit polyclonal antibody to Gpx6 Covance (Denver, PA). Two polyclonal antibodies have been raised in rabbit hosts, each targeting the Gpx6-specific peptide "SDIMEYLNQ" (Seq ID No: 1) The antibodies are peptide affinity purified.

[00134] Table 1. Genes with significant changes (Benjamini-Hochberg adjusted p-values < 0.05) of at least 1.2-fold up or down in *Drd1a*-expressing striatal medium spiny neurons at 2 years and 6 weeks of age, as compared to 6 weeks of age.

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene Symbol
6899520 20194	0.04976691	5.15E-04	2.8210127	up	S100a10
7023132 236604	0.003987592	5.61E-07	2.3643906	up	Piscd-ps3 Piscd-ps1
6761825 269109	0.003987592	3.56E-07	2.3027277	up	Dpp10
6981113 83436	0.031307697	1.72E-04	2.278604	up	Plekha2
6886678 74194	0.020062922	5.50E-05	2.2697477	down	Rnd3
6841712 320712	0.013143726	1.24E-05	2.2293866	up	Abi3bp
6850534 27226	0.007663706	5.02E-06	2.1952941	up	Pla2g7
6753402 21956	0.007663706	5.44E-06	2.1893516	up	Tunt2
6777510 73914	0.014433213	1.93E-05	2.15296	up	Irak3
7013389 237010	0.015460879	3.15E-05	2.1512105	up	Klhl4
6768075 12140	0.026665783	1.07E-04	2.069984	down	Fabp7
6880670 12010	0.005005356	1.24E-06	2.0678577	up	B2m
7017520 14396	0.024065567	9.01E-05	2.0567203	up	Gabra3
6967593 110886	0.034732584	2.22E-04	2.0417027	down	Gabra5
6861441 328971	0.016631078	3.70E-05	2.0326183	down	Spink10
6860204 93890	0.017962048	4.52E-05	2.0307233	down	Pcdhb19
6764133 18611	0.005005356	1.92E-06	2.0271978	up	Pea15a
6900456 57257	0.024065567	8.94E-05	1.9663367	up	Vav3
6957263 12444	0.007264868	4.09E-06	1.9645411	down	Ccnd2
6937190,702313 2 320951	0.005005356	2.30E-06	1.9601252	up	Piscd Piscd-ps3//Piscd-ps3 Piscd-ps1
6937190,702313 2 66776	0.005005356	2.30E-06	1.9601252	up	Piscd Piscd-ps3//Piscd-ps3 Piscd-ps1
6768479 13654	0.034732584	2.22E-04	1.9502792	down	Egr2
6860170 93877	0.04976691	4.83E-04	1.9439118	up	Pcdhb6
6869570 74055	0.005005356	2.46E-06	1.9408888	up	Plec1
6824507 67419	0.039366398	2.78E-04	1.8852826	up	3632451O06Rik
6919895 69352	0.013143726	1.57E-05	1.859933	up	Necab1
6953887 18575	0.031307697	1.66E-04	1.8485277	up	Pde1c
6919417 252838	0.014433213	2.52E-05	1.8419087	up	Tox
6879646 12509	0.042573277	3.29E-04	1.7996722	up	Cd59a Cd59b
6879646 333883	0.042573277	3.29E-04	1.7996722	up	Cd59a Cd59b
6758223 66297	0.007663706	5.93E-06	1.7777925	down	2610017I09Rik
6805200 75512	0.013143726	1.55E-05	1.7739272	up	Gpx6
6919304 56711	0.04630302	4.23E-04	1.7644565	up	Plagl1
7014941 55936	0.014433213	2.28E-05	1.7513621	up	Ctps2
6766409 52906	0.014433213	2.54E-05	1.7504913	up	Ahil
6832146 105859	0.024065567	8.76E-05	1.7445399	up	Csdc2
6791494 73635	0.013143726	1.39E-05	1.735179	down	Rundc1 1700113I22Rik Aarsd1
6830852,683607 9 320469	0.016631078	3.86E-05	1.7265527	down	9930014A18Rik Fam84b//Fam84b 9930014A18Rik
6830852,683607 9 399603	0.016631078	3.86E-05	1.7265527	down	9930014A18Rik Fam84b//Fam84b 9930014A18Rik
6837848 54526	0.047644805	4.56E-04	1.7124188	up	Syt10
6805383,681169 7 319148	0.014433213	2.18E-05	1.708725	down	Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3h Hist1h3i//Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3f Hist1h3g Hist1h3h Hist1h3i
6860188 93885	0.024065567	8.95E-05	1.7023137	up	Pcdhb14

6989222 12903	0.027466808	1.16E-04	1.7015634	down	Crabp1
6834890 56274	0.024065567	8.70E-05	1.6866167	up	Stk3
6784587 11421	0.017962048	4.80E-05	1.6859602	down	Ace Ace3
6784587 217246	0.017962048	4.80E-05	1.6859602	down	Ace Ace3
6843811 74720	0.043797355	3.74E-04	1.6742324	down	Tmem114
7015229 11856	0.041084405	3.00E-04	1.66966	up	Arhgap6
6908528 114301	0.039366398	2.81E-04	1.6680608	down	Palmd
6809522 20365	0.013143726	1.37E-05	1.6649965	down	Serf1
6838460 72393	0.024065567	7.84E-05	1.6563784	up	Faim2
6978855 56513	0.03619993	2.44E-04	1.6498939	down	Pard6a
6869068 77125	0.04976691	5.03E-04	1.645941	up	H33
6768261,687613 8 432466	0.031307697	1.75E-04	1.6448121	up	Gm5424 Ass1 Ass1 Gm5424
6768261,687613 8 11898	0.031307697	1.75E-04	1.6448121	up	Gm5424 Ass1 Ass1 Gm5424
6792679 30951	0.015102888	2.80E-05	1.6424714	down	Cbx8
6759997 20254	0.015102888	2.87E-05	1.6344112	up	Scg2
6955137 94282	0.023366889	7.06E-05	1.6341208	down	Sfxn5
6781933 276920	0.04635039	4.37E-04	1.6271018	up	Ccdc42
6833311 15370	0.04976691	5.26E-04	1.6136416	down	Nr4a1
6805360 319181	0.031008814	1.50E-04	1.6091015	down	Hist1h2bg
6799173 217410	0.021131802	6.24E-05	1.6087055	down	Trib2
6850191 15937	0.021131802	6.23E-05	1.6082655	up	Ier3
6954572 104263	0.031335603	1.87E-04	1.6013699	up	Kdm3a
6903983 241919	0.008627407	7.28E-06	1.5909182	up	Slc7a14
6874631 16922	0.039366398	2.82E-04	1.577109	up	Phyh
6755757 72978	0.043715313	3.67E-04	1.5758797	down	Cnih3
6805255,680527 3,6805370 6802 4	0.031335603	1.79E-04	1.573402	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646
6756790 17864	0.017962048	4.55E-05	1.5719444	up	Mybl1
6834108 12563	0.025328478	9.97E-05	1.5705953	down	Cdh6
6815437 238799	0.042669825	3.37E-04	1.5662972	up	Tnpo1
6969021 11864	0.014433213	2.43E-05	1.5643754	up	Arnt2
6900404 99730	0.024065567	7.75E-05	1.5643417	down	Taf13
6805255,680527 3,6805370,6811 533 665622	0.031307697	1.76E-04	1.5641509	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6805255,680527 3,6805370,6811 533 665596	0.031307697	1.76E-04	1.5641509	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6805255,680527 3,6805370,6811 533 319183	0.031307697	1.76E-04	1.5641509	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6805237,680535 7,6805383,6811 531,6811697,68 11702 319152	0.027739117	1.27E-04	1.5599499	down	Hist1h3h Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3i Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3b Hist1h3i Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3h Hist1h3i Hist1h3i Hist1h3b Hist1h3d Hist1h3e H

					ist1h3g Hist1h3h Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3f Hist1h3h Hist1h3i Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3h Hist1h3i
6791641 14580	0.024065567	7.86E-05	1.5587983	up	Gfap
6805237,6805357,6805364,6805383,6811531,6811697,6811702 319150	0.042669825	3.51E-04	1.5567741	down	Hist1h3h Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3i Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3b Hist1h3d Hist1h3e Hist1h3i Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3h Hist1h3i Hist1h3i Hist1h3b Hist1h3d Hist1h3e Hist1h3e Hist1h3g Hist1h3h Hist1h3b Hist1h3e Hist1h3d Hist1h3e Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3h Hist1h3i
6805237,6805357,6805364,6805383,6811531,6811697,6811702 319149	0.042669825	3.51E-04	1.5567741	down	Hist1h3h Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3i Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3b Hist1h3d Hist1h3e Hist1h3i Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3h Hist1h3i Hist1h3i Hist1h3b Hist1h3d Hist1h3e Hist1h3e Hist1h3g Hist1h3h Hist1h3b Hist1h3e Hist1h3d Hist1h3e Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3h Hist1h3i
6805237,6805357,6805364,6805383,6811531,6811697,6811702 319153	0.042669825	3.51E-04	1.5567741	down	Hist1h3h Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3i Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3b Hist1h3d Hist1h3e Hist1h3i Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3h Hist1h3i Hist1h3i Hist1h3b Hist1h3d Hist1h3e Hist1h3e Hist1h3g Hist1h3h Hist1h3b Hist1h3e Hist1h3d Hist1h3e Hist1h3a Hist1h3b Hist1h3d Hist1h3e Hist1h3g Hist1h3h Hist1h3i
6805255,6805270,6805273,6805370,6811533 319184	0.031335603	1.87E-04	1.555105	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bk Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6971344 66422	0.025328478	9.94E-05	1.5520357	down	Detpp1
6905746 17035	0.043715313	3.68E-04	1.5490541	up	Lxn
6764526 69726	0.04630302	4.30E-04	1.5490206	up	Smyd3
6778528 56418	0.027466808	1.16E-04	1.5428835	down	Ykt6
6769343,6773537,6968533 624784	0.014433213	2.54E-05	1.5391593	down	Tdg Gm9855 Gm5806
6769343,6773537,6968533 545124	0.014433213	2.54E-05	1.5391593	down	Tdg Gm9855 Gm5806
6805358,6811681,6811697 260423	0.017464902	4.17E-05	1.5370511	down	Hist1h3i Hist1h3e Hist1h3b Hist1h3c Hist1h3d Hist1h3e Hist1h3f Hist1h3h Hist1h3i
6785684 380684	0.031875465	1.93E-04	1.5352144	up	Nefh
6977139 326618	0.024065567	9.03E-05	1.5304857	down	Tpm4
6972181 15461	0.021131802	6.21E-05	1.5240446	down	Hras1
6910642 229949	0.04635039	4.34E-04	1.5222185	up	Ak5
6769343,6773537,6775518,6968533 21665	0.015460879	3.15E-05	1.518443	down	Tdg Gm9855 Gm5806 Glt8d2 Tdg



6866919 68731	0.04196097	3.18E-04	1.3859518	down	I110032A13Rik
6855669 75564	0.04597941	4.01E-04	1.3830876	up	Rsph9
6795889 238247	0.04196097	3.10E-04	1.3828267	up	Arid4a
6754526 73844	0.039366398	2.82E-04	1.3790938	up	Ankrd45
6845559 76916	0.04976691	5.07E-04	1.3761423	down	4930455C21Rik
6881306 110911	0.04550051	3.93E-04	1.358163	up	Cds2
6916947 170638	0.041004203	2.97E-04	1.3532506	up	Hpcal4
6823724 67011	0.042669825	3.42E-04	1.3496869	down	Mettl6
6787525 14406	0.04630302	4.29E-04	1.3473492	up	Gabrg2
6845459 207227	0.04976691	5.31E-04	1.3440369	up	Stxbp5l
6765596 66084	0.04976691	5.22E-04	1.3427882	down	Rrund1 Gm5512
6765596 433224	0.04976691	5.22E-04	1.3427882	down	Rmad1 Gm5512
6881100,688110 1 78751	0.04630302	4.10E-04	1.3219867	up	Zc3h6

**[00135]** Table 2. Genes with significant changes (Benjamini-Hochberg adjusted p-values < 0.05) of at least 1.2-fold up or down in *Drd2*-expressing striatal medium spiny neurons at 2 years and 6 weeks of age, as compared to 6 weeks of age.

D2 Striatum.txt

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene symbol
6813284 13488	1.10E-04	7.73E-09	5.3255243	up	<i>Drd1a</i>
6860176 93879	1.40E-04	1.18E-07	4.5493364	up	<i>Pcdhb8</i>
6880670 12010	5.82E-04	1.15E-06	4.148419	up	<i>B2m</i>
6879087 12672	1.40E-04	1.06E-07	3.5000043	up	<i>Chrm4</i>
6877229 16519	1.40E-04	1.15E-07	3.1247575	up	<i>Kcnj3</i>
6905530 229357	2.28E-04	2.41E-07	3.0322802	up	<i>Gpr149</i>
6747641 240725	2.28E-04	2.44E-07	2.9879596	up	<i>Sulf1</i>
6764133 18611	4.32E-04	6.07E-07	2.9562507	up	<i>Pea15a</i>
6998397 22041	0.007923391	5.85E-05	2.8957565	up	<i>Trf</i>
6845079 11815	0.008547507	6.73E-05	2.7908227	up	<i>Apod</i>
6761825 269109	0.003589682	1.74E-05	2.7531443	up	<i>Dpp10</i>
6805200 75512	0.003589682	1.73E-05	2.7515676	up	<i>Gpx6</i>
6886678 74194	0.015283823	2.01E-04	2.741378	down	<i>Rnd3</i>
6748020 14859	0.003589682	1.70E-05	2.643068	up	<i>Gsta3</i>
6943974 21333	0.003656822	1.83E-05	2.6233518	up	<i>Tac1</i>
6834890 56274	0.002267633	7.65E-06	2.579052	up	<i>Stk3</i>
6791494 73635	0.00779995	5.70E-05	2.5706615	down	<i>Runde1 1700113122Rik Aarsd1</i>
6835759 18606	0.004721215	2.63E-05	2.5419888	up	<i>Enpp2</i>
6776577 67405	0.020587178	4.19E-04	2.534108	down	<i>Nts</i>
6767537,6822154 12484	0.002235683	7.23E-06	2.5077183	down	<i>Cd24a</i>
6824610 29811	0.004966004	2.93E-05	2.4164193	up	<i>Ndrg2</i>
6917180 269582	3.81E-04	5.09E-07	2.3707016	down	<i>Clspn</i>
7023132 236604	1.34E-04	3.86E-08	2.3301787	up	<i>Pisd-ps3 Pisd-ps1</i>
6811068 56048	0.001255512	3.09E-06	2.2832966	up	<i>Lgals8</i>
6860170 93877	1.40E-04	8.96E-08	2.2796516	up	<i>Pcdhb6</i>
6841712 320712	6.50E-04	1.42E-06	2.2767649	up	<i>Abi3bp</i>

6753402 21956	2.28E-04	2.57E-07	2.1717684	up	Tnnt2
6819244 12891	5.82E-04	9.67E-07	2.1581087	up	Cpne6
7013389 237010	0.005432868	3.59E-05	2.1391268	up	Klhl4
6908078,6908079 14862	0.001185273	2.76E-06	2.1385758	up	Gstm1 Gstm3///Gstm2 Gstm1
6838460 72393	0.002798558	1.18E-05	2.1031454	up	Faim2
6855981 20230	1.34E-04	7.55E-08	2.0987513	down	Satb1 5830444F18Rik C230085N15Rik E430014B02Rik
6855981 320804	1.34E-04	7.55E-08	2.0987513	down	Satb1 5830444F18Rik C230085N15Rik E430014B02Rik
6855981 320556	1.34E-04	7.55E-08	2.0987513	down	Satb1 5830444F18Rik C230085N15Rik E430014B02Rik
6855981 320908	1.34E-04	7.55E-08	2.0987513	down	Satb1 5830444F18Rik C230085N15Rik E430014B02Rik
6805381 50708	0.02441559	6.11E-04	2.0501385	down	Hist1h1c
6869068 77125	5.88E-04	1.24E-06	2.0441618	up	Il33
6807154 14057	0.004926001	2.84E-05	2.0316029	up	Sfxn1
6805360 319181	2.63E-04	3.14E-07	2.0225863	down	Hist1h2bg
6815345 15212	0.014639024	1.65E-04	2.0052912	up	Hexb
6937190,7023132 320951	1.34E-04	5.77E-08	2.003108	up	Pisd Pisd-ps3///Pisd-ps3 Pisd-ps1
6937190,7023132 66776	1.34E-04	5.77E-08	2.003108	up	Pisd Pisd-ps3///Pisd-ps3 Pisd-ps1
6974682 320158	0.001867071	5.38E-06	1.9956818	down	Zmat4
6798951 26950	0.01630344	2.40E-04	1.9872415	up	Vsnl1
6936702 84652	0.00156325	4.29E-06	1.9783112	up	Fam126a
6994887 72828	0.015052847	1.77E-04	1.9770834	down	Ubash3b
6996956 20255	0.002493485	9.27E-06	1.9685587	up	Seg3
6860188 93885	0.002235683	7.21E-06	1.9678934	up	Pcdhb14
6800468 217517	0.001915143	5.66E-06	1.9609915	up	Stxbp6
7015648 71458	0.008480565	6.56E-05	1.9547465	down	Bcor
6805255,6805273,6805370 68024	5.82E-04	1.08E-06	1.9373631	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm///Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646///Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646
6908075,6908077,6908078 14864	0.003549275	1.62E-05	1.9293586	up	Gstm6 Gstm3///Gstm3///Gstm1 Gstm3
6959584 22177	0.042509187	0.001670174	1.926382	up	Tyrobp
6882307 66405	0.001386939	3.61E-06	1.923774	down	Mcts2
6805255,6805273,6805370,6811533 665622	5.82E-04	1.12E-06	1.9198099	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm///Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646///Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646///Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6805255,6805273,6805370,6811533 665596	5.82E-04	1.12E-06	1.9198099	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm///Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646

					77 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6805255,6805273,6805370,6811533 319183	5.82E-04	1.12E-06	1.9198099	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6973587 11816	0.040252663	0.001514109	1.9158078	up	Apoe
6899520 20194	8.42E-04	1.89E-06	1.913387	up	Sl100a10
6805255,6805270,6805273,6805370,6811533 319184	5.88E-04	1.20E-06	1.9044812	down	Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Hist1h2bl Hist1h2bm Hist1h2bk Hist1h2bj Hist1h2bc Hist1h2bk Gm11277 Gm13646 Hist1h2bc Hist1h2bj Hist1h2bk Gm11277 Gm13646 Gm11277 Gm13646 Hist1h2bj Hist1h2bk
6880467 214240	0.003312399	1.49E-05	1.9037254	up	Disp2
6827410 76965	0.003589682	1.72E-05	1.8947399	up	Slit1rkl
6780443 13591	0.004721215	2.66E-05	1.8885926	up	Ebfl
6928871 20346	1.65E-04	1.50E-07	1.8852962	up	Sema3a
6944262 114142	2.98E-04	3.77E-07	1.8727168	up	Foxp2
6883533 76829	0.013542953	1.44E-04	1.8723825	down	Dok5
6930606 20563	0.031038841	9.43E-04	1.8607157	up	Slit2 Mir218-1
6930606 723822	0.031038841	9.43E-04	1.8607157	up	Slit2 Mir218-1
7002980,7004901,7005644,7006456 12047	0.01798404	2.89E-04	1.8528872	up	Bcl2a1d Bcl2a1b Bcl2a1a Bcl2a1a Bcl2a1c Bcl2a1d Bcl2a1b
7002980,7004901,7005644,7006456 12045	0.01798404	2.89E-04	1.8528872	up	Bcl2a1d Bcl2a1b Bcl2a1a Bcl2a1a Bcl2a1c Bcl2a1d Bcl2a1b
7002980,7004901,7005644,7006456 12044	0.01798404	2.89E-04	1.8528872	up	Bcl2a1d Bcl2a1b Bcl2a1a Bcl2a1a Bcl2a1c Bcl2a1d Bcl2a1b
6874631 16922	0.01798404	2.91E-04	1.8519856	up	Phyh
6864444 170459	0.001735423	4.88E-06	1.833533	up	Stard4
6772476 76157	0.026050128	6.84E-04	1.8292406	up	Slc35d3
6756637 58175	0.043834306	0.001858774	1.8150766	down	Rgs20
7017520 14396	0.038419306	0.001399085	1.8125371	up	Gabra3
6863973 106957	0.002037618	6.16E-06	1.809555	up	Slc39a6
6880931 26458	0.04682665	0.002093915	1.8083715	up	Slc27a2
6940611 13602	0.002789888	1.12E-05	1.8027624	up	Spare1 Scpppq1
6940611 100271704	0.002789888	1.12E-05	1.8027624	up	Spare1 Scpppq1
6989100 19684	0.013600663	1.47E-04	1.7838393	up	Rdx
6820055 13655	0.019556254	3.55E-04	1.7764342	down	Egr3
6897908 18441	0.002536604	9.63E-06	1.7762277	up	P2ry1
6990685 14860	0.021159004	4.42E-04	1.7742459	up	Gsta4



6869570 74055	0.004721215	2.62E-05	1.7690808	up	Plec1
6916947 170638	0.002319411	8.15E-06	1.76002	up	Hpcal4
6949160 74244	0.011257361	1.08E-04	1.7584462	up	Atg7 LOC100043926
6949160 1000439 26	0.011257361	1.08E-04	1.7584462	up	Atg7 LOC100043926
7000764 77226	0.03287614	0.001040165	1.7505	down	9330169L03Rik
6884986 74103	0.019693213	3.82E-04	1.7502115	down	Nebi
6754867 226610	0.00430831	2.33E-05	1.7439637	down	Fam78b
6756985 72265	0.015268379	2.00E-04	1.7430842	up	Tram1
6816708 67053	0.0332504	0.001087987	1.7329823	down	Rpp14
6862062 71263	0.015283823	2.02E-04	1.7212113	down	Mro
6913009,6921154  12517	0.001302391	3.30E-06	1.7166166	down	Tesk1 Cd72///Cd72
6900404 99730	0.019556254	3.71E-04	1.706687	down	Taf13
6813560 56278	0.0281969	8.04E-04	1.7064552	up	Gkap1
6908075 14867	0.049958326	0.002381477	1.7012932	up	Gstm6 Gstm3
7011393 236794	0.023736937	5.72E-04	1.6997313	up	Slc9a6
6948759 12661	0.006945028	4.93E-05	1.6881636	up	Chl1
6954385 13197	0.020429397	4.11E-04	1.6871984	down	Gadd45a Gng12
6861689 67064	0.011394512	1.10E-04	1.6851403	down	Chmp1b
6799173 217410	0.017824696	2.74E-04	1.6835176	down	Trib2
6763146 74091	0.027805798	7.86E-04	1.6814463	down	Npl
6790317 56405	0.022105824	4.82E-04	1.6790038	down	Dusp14
6845978 17470	0.04312894	0.001749659	1.6726958	up	Cd200
6791641 14580	0.011568548	1.12E-04	1.6671637	up	Gfap
6754138 19734	0.033966344	0.001124808	1.6642561	up	Rgs16
6763991 19736	0.005185398	3.14E-05	1.6637514	down	Rgs4
6778939 211739	0.010969274	9.82E-05	1.6624225	up	Vstm2a Hmgbl
6782694 11676	0.044839386	0.001942	1.6547385	up	Aldoc
6957263 12444	0.011084057	1.02E-04	1.6481607	down	Ccnd2
6987109 14608	0.029066546	8.46E-04	1.6443005	up	Gpr83
7015229 11856	0.002298735	7.92E-06	1.6439329	up	Arhgap6
6898630 68659	0.032440964	0.001005868	1.6438571	down	Fam198b
6768155 19156	0.005023014	3.00E-05	1.6399189	up	Psap
6784371 73293	0.019556254	3.71E-04	1.6378373	down	Ccdc103 4933439F11Rik
6784371 66784	0.019556254	3.71E-04	1.6378373	down	Ccdc103 4933439F11Rik
6854453 224624	0.010969274	9.66E-05	1.6355382	down	Rab40c
6946412 11517	5.82E-04	1.09E-06	1.6319461	up	Adcyap1r1
6758223 66297	0.010677658	9.23E-05	1.6314174	down	2610017I09Rik
6961010 17984	0.04115921	0.001591617	1.6312153	up	Ndn
6748437 170771	0.00285053	1.24E-05	1.6293082	up	Khdrbs2
6824507 67419	0.026061453	6.89E-04	1.6290909	up	3632451O06Rik
6816288 16392	0.047316674	0.002138365	1.6269062	up	Isl1
6823068 11750	0.015052847	1.84E-04	1.6259166	up	Anxa7
6916089 74754	0.01608881	2.28E-04	1.625083	up	Dhcr24
7020407 18675	0.013542953	1.46E-04	1.6249138	down	Phex
6869635 12495	0.01811096	2.99E-04	1.6211282	down	Entpd1 Tctn3
6876072 78617	0.036739744	0.001298216	1.6201752	down	Cstad
6963558 11865	5.19E-04	7.66E-07	1.6189378	down	Arntl
6866643 107029	0.020970276	4.31E-04	1.6186217	down	Me2
6864327 20983	0.008480565	6.43E-05	1.6172807	up	Syl4
6872616 19091	0.047316674	0.002129742	1.6146805	up	Prkg1
7018897 50887	0.049103312	0.0023131	1.6131068	up	Hmgn5

6753397 21952	0.004729526	2.69E-05	1.6111035	down	Tnni1
6895790 76897	0.045339916	0.001979617	1.6094204	up	Raly1
6749115 70676	0.037047874	0.001323231	1.6076359	up	Gulp1
6912565 12801	0.011134457	1.03E-04	1.6063108	down	Cnr1
6916220 69908	0.018251646	3.04E-04	1.6042217	up	Rab3b
6981113 83436	0.02097058	4.33E-04	1.6026766	up	Plekha2
6878655 16410	0.012477017	1.23E-04	1.6024555	up	Itgav
6766409 52906	0.00156325	4.29E-06	1.5939846	up	Ahi1
6777286 216363	0.016230881	2.33E-04	1.5891256	down	Rab3ip
6976395 234290	0.011135913	1.05E-04	1.5849389	down	BC030500
6834558 432940	0.004089114	2.16E-05	1.5849028	down	Fam105b
6769343,6773537, 6968533 624784	0.002493485	9.29E-06	1.5830903	down	TdgiGm9855 Gm5806
6769343,6773537, 6968533 545124	0.002493485	9.29E-06	1.5830903	down	TdgiGm9855 Gm5806
6784345 14824	0.002267633	7.52E-06	1.5818839	up	Grn
6842273 74185	0.02970668	8.71E-04	1.578027	down	Gbel
6832146 105859	0.005263386	3.29E-05	1.5721171	up	Csdc2
6769445 216198	0.043429643	0.001801553	1.5719231	up	Tcp112
6878702 241525	0.04797561	0.002202635	1.5712873	up	Ypel4
6829659 17181	0.001185273	2.83E-06	1.57119	up	Matn2
6877356,6886947  77767	0.029997475	8.86E-04	1.5697238	up	Galnt5 Ernn//Ernn
6829123 215654	0.04815002	0.002240778	1.5685539	up	Cdh12
6791528 72349	0.04337046	0.001785073	1.5679616	down	Dusp3
6812770 67046	0.025499985	6.60E-04	1.5675546	down	Tbcd7
6966198 20733	0.01608881	2.26E-04	1.5670083	up	Spint2
6892747 19281	0.015322137	2.05E-04	1.5650489	up	Ptprt
7016726 236781	0.002798558	1.15E-05	1.5631052	down	Gpr119
6916219 100087	0.017905615	2.77E-04	1.5617313	down	Kti12
6871297 70999	0.035202216	0.001204654	1.561103	down	Naa40
6995454 17967	0.01394069	1.54E-04	1.5575242	up	Ncam1
6769343,6773537, 6775518,6968533  21665	0.002537387	9.81E-06	1.5570186	down	TdgiGm9855 Gm5806//Glt8 d2 Tdg
6876570 74192	0.010677658	9.23E-05	1.550878	down	Arpe5l
6764721 12334	0.04193666	0.001627578	1.5500118	up	Capn2
6997114 235504	0.018667279	3.19E-04	1.5480542	up	Slc17a5
6768261,6876138  432466	0.014639024	1.67E-04	1.5479015	up	Gm5424 Ass1//Ass1 Gm542 4
6768261,6876138  11898	0.014639024	1.67E-04	1.5479015	up	Gm5424 Ass1//Ass1 Gm542 4
6877954 329421	0.04884973	0.00229772	1.5478375	down	Myo3b
6864518 75533	0.04031455	0.001524941	1.5474952	up	Nme5
6932930 74596	0.015249936	1.97E-04	1.546657	up	Cds1
6761256 12043	0.028586583	8.22E-04	1.5446783	down	Bcl2
6946339,6953749  69993	0.022382699	5.04E-04	1.5439757	up	Chn2 9130019P16Rik//9130 019P16Rik Chn2
6946339,6953749  100042056	0.022382699	5.04E-04	1.5439757	up	Chn2 9130019P16Rik//9130 019P16Rik Chn2
6784266,6791494  217201	0.008480565	6.49E-05	1.5408274	down	Runde1//Runde1 1700113I2 2Rik Aarsd1
6972294 13033	0.044557586	0.001923529	1.5388831	up	Ctsd

6786045 13195	0.015820324	2.16E-04	1.5372787	up	Ddc
6780961 67966	0.01608881	2.28E-04	1.5367461	down	Zcchc10
6825705 20389	0.015137184	1.92E-04	1.5347135	down	Sftpc
6762321 381290	0.02575794	6.68E-04	1.532662	up	Atp2b4
6770201 17311	0.012557453	1.24E-04	1.5317988	down	Kitl
6993067 67469	0.020444345	4.13E-04	1.5317638	down	Abhd5
6815511 27220	0.016510215	2.48E-04	1.52917	up	Cartpt
6754149,6861135 14645	0.018376803	3.09E-04	1.5289168	up	Glu1///Grand3 Glu1
7011581 331424	0.010673828	8.84E-05	1.5289078	down	C230004F18Rik C030023E24Rik
7011581 320247	0.010673828	8.84E-05	1.5289078	down	C230004F18Rik C030023E24Rik
6803780 67236	0.010677658	9.00E-05	1.5288949	down	Cinp
6998305 235542	0.03324496	0.001069841	1.5287542	up	Ppp2r3a
6800314 16981	0.005284981	3.34E-05	1.5286509	up	Lrrn3
6972181 15461	0.005295044	3.39E-05	1.5272726	down	Hras1
6808621 723967	0.003656822	1.85E-05	1.5243323	down	Mir9-2 C130071C03Rik
6808621 320203	0.003656822	1.85E-05	1.5243323	down	Mir9-2 C130071C03Rik
6988855 54725	0.04953374	0.002343567	1.5239736	up	Cadm1
6878548 68082	0.038419306	0.001394552	1.5217838	down	Dusp19
6768910 20203	0.021932513	4.73E-04	1.5203797	up	S100b
6948964 108073	0.002072472	6.41E-06	1.5164479	up	Grm7
6949826 30853	0.01984823	3.93E-04	1.5146208	down	Mlf2
6861751 52662	0.008547507	6.72E-05	1.5145016	down	D18Ertid653e
7014941 55936	0.019081173	3.30E-04	1.5129799	up	Ctps2
6750314 320460	0.030360658	9.05E-04	1.5119076	up	Vwe2l
6964557 66885	0.0332504	0.001078375	1.5105767	up	Acadslb
6885395 68475	0.022202644	4.90E-04	1.5084188	down	Ssna1
6933679 77407	0.005520782	3.69E-05	1.5056041	down	Rab35
6779845 327900	0.019560797	3.77E-04	1.502835	down	Ubtid2
6969021 11864	0.00430831	2.33E-05	1.502225	up	Arnt2
6988958 235323	0.002835888	1.22E-05	1.5014262	down	Usp28
6791230 217151	0.019556254	3.56E-04	1.5006561	down	Arl5c
6854276 76917	0.015052847	1.85E-04	1.4991108	down	Flyweb2
6899747,6907247 15267	0.024054471	5.85E-04	1.497548	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1///Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6899747,6907247 319192	0.024054471	5.85E-04	1.497548	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1///Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6973739 20300	0.033489518	0.001101954	1.4958638	down	Ccl25
6916708 80509	0.018667279	3.19E-04	1.4954613	down	Med8
6955034 27369	0.00312503	1.38E-05	1.4942619	down	Dguok
6881306 110911	0.002798558	1.17E-05	1.4928799	up	Cds2
6937364 16976	0.02366127	5.51E-04	1.4919764	up	Lrpap1
6824195 70561	0.021932513	4.71E-04	1.4907249	up	Txndc16
6869932,6873271 20250	0.005263386	3.28E-05	1.490423	up	Scd2 Scd1///Scd1 Scd2
6869932,6873271 20249	0.005263386	3.28E-05	1.490423	up	Scd2 Scd1///Scd1 Scd2
6891493 71436	0.021438045	4.52E-04	1.4899818	up	Flrt3
6780844 619293	0.01394069	1.53E-04	1.4898849	down	Zfp354a Zfp354b 923000910

						2Rik
6982921 66234	0.011613366	1.13E-04	1.4868916	up		Sc4mol
6972256 101513	0.034334507	0.001144242	1.4851689	down		2700078K21Rik
6990673 68801	0.019556254	3.70E-04	1.4836878	up		Elovl5
6831709 117171	0.02606873	6.94E-04	1.4806932	down		1110038F14Rik
6869436 226098	0.036910944	0.00131315	1.4780036	down		Hectd2
6803269 71907	0.039544	0.001466365	1.4775524	up		Serpina9
6891905 13010	0.047668647	0.002171784	1.47457	up		Cst3
6838469 26934	0.015052847	1.81E-04	1.4744074	up		Raegap1
6933491 330164	0.04208484	0.00163976	1.4724283	down		C130026L21Rik
6937522 22393	0.0281969	8.09E-04	1.470271	up		Wfs1
6784412 57778	0.015052847	1.78E-04	1.4695581	down		Fmnl1
6903983 241919	0.015137184	1.90E-04	1.4690902	up		Slc7a14
6918814 65945	0.004966004	2.90E-05	1.4689643	up		Clstn1
6928759 231014	0.02585882	6.73E-04	1.4681538	up		9330182L06Rik
6933616,6941218 68420	0.018114181	3.01E-04	1.4663692	down		Ankrd13a//4930515G01Rik Ankrd13a
6808997 26556	0.004002512	2.08E-05	1.4656779	down		Homer1 C330006P03Rik
6808997 320588	0.004002512	2.08E-05	1.4656779	down		Homer1 C330006P03Rik
6789325 12514	0.022007378	4.78E-04	1.4655061	down		Cd68
6902665 209601	0.015204828	1.95E-04	1.4653959	up		4922501L14Rik
6863645 12558	0.030360658	9.02E-04	1.4628594	up		Cdh2
6837805 77980	0.020587178	4.20E-04	1.4586661	up		Sbfl
6980052 16337	0.016593723	2.51E-04	1.4583049	up		Insr
6990244 235459	0.022598844	5.20E-04	1.4577506	down		Gtf2a2
6957119 14791	0.015983123	2.21E-04	1.4573512	down		Emg1 Lpcat3
6766705 13822	0.024054471	5.87E-04	1.4570173	down		Epb4.1l2
6880972 109778	0.013242392	1.36E-04	1.4568212	up		Blvra
6752222 241201	0.035368353	0.001219077	1.4561962	up		Cdb7
6803136 110616	0.031045154	9.45E-04	1.4553119	up		Atxn3
6771581 21334	0.022202644	4.93E-04	1.4538059	up		Tac2
6866486 80718	0.015052847	1.77E-04	1.453329	down		Rab27b
6989438 20361	0.021438045	4.51E-04	1.453112	down		Sema7a
6885872 73737	0.00533197	3.45E-05	1.4524046	down		1110008P14Rik
6969818 27276	0.027616503	7.63E-04	1.4516916	up		Plekhl1
6956748 67784	0.016593723	2.52E-04	1.4502109	up		Plxnd1
6791995 71795	0.006391116	4.31E-05	1.4501014	down		Pitpnc1
7012006 54411	0.028827934	8.33E-04	1.4465153	up		Atp6ap1
6858134 18189	0.03380979	0.001117247	1.446442	up		Nrxn1
6801507 94090	0.019246986	3.41E-04	1.4461541	down		Trim9
6768151 94214	0.015204828	1.95E-04	1.4460502	up		Spock2
6938891 11980	0.020970276	4.32E-04	1.4438521	up		Atp8a1
6843340 70028	0.04115921	0.001580126	1.4437007	up		Dopey2
6929762 277854	0.019152917	3.35E-04	1.4435827	up		Depdc5
6950397,6957687 74525	0.01601894	2.23E-04	1.4433552	up		8430419L09Rik//Gsg1 8430419L09Rik
6806444 66154	0.017905615	2.80E-04	1.4423473	down		Tmem14c
6838257 67760	0.015268379	1.99E-04	1.4420997	up		Slc38a2
6949992 101187	0.032736823	0.001031154	1.4404699	down		Parp1
6801807 238271	0.029657012	8.67E-04	1.4399031	up		Kcnnh5
6785684 380684	0.01910948	3.32E-04	1.4397109	up		Nefh
6792994 382562	0.013328801	1.39E-04	1.4389725	down		Pfn4
6986775 22068	0.024526443	6.19E-04	1.4386616	down		Trpc6

6769934 77048	0.006945028	4.92E-05	1.4383348	down	Ccdc41
6785367 14387	0.032434884	9.99E-04	1.4367542	up	Gaa
6767850 215085	0.028827934	8.33E-04	1.4356312	up	Slc35f1
6845139 106264	0.020325309	4.07E-04	1.4345336	down	0610012G03Rik
6778528 56418	0.037560377	0.001344177	1.434402	down	Ykt6
6830852,6836079 320469	0.043119576	0.001743216	1.4332331	down	9930014A18Rik Fam84b///Fam84b 9930014A18Rik
6830852,6836079 399603	0.043119576	0.001743216	1.4332331	down	9930014A18Rik Fam84b///Fam84b 9930014A18Rik
6750557 66821	0.02343562	5.44E-04	1.4325122	down	Bcs1 Zfp142
6885924 99326	0.017736405	2.72E-04	1.4325033	down	Garnl3
6831469 19245	0.029353406	8.56E-04	1.4322174	down	Ptp4a3
6904979 73251	0.022598844	5.19E-04	1.4321386	down	Setd7
6898477 20713	0.022454733	5.10E-04	1.4302071	up	Serpini1
6844567 110197	0.01916445	3.37E-04	1.4295702	down	Dgkg
6960328 20130	0.048653852	0.002277486	1.4291523	down	Rras
6754893 56752	0.02606873	6.96E-04	1.42853	up	Aldh9a1
6780882 52626	0.04815002	0.002239924	1.426755	up	Cdkn2aipnl
6791212 22658	0.012751671	1.29E-04	1.4259104	up	Pcgf2
6838171 54003	0.044442587	0.001914528	1.423863	up	Nell2
6823302 71228	0.029997475	8.84E-04	1.4204878	up	Dlg5
6829598 15529	0.019556254	3.65E-04	1.4202565	up	Sdc2
6878511 66861	0.015052847	1.81E-04	1.4187359	up	Dnajc10
6821431,6989873 50933	0.020308778	4.06E-04	1.4180315	down	Uchl3 Uchl4///Uchl4 Uchl3
6821431,6989873 93841	0.020308778	4.06E-04	1.4180315	down	Uchl3 Uchl4///Uchl4 Uchl3
6952523 243743	0.028027382	7.96E-04	1.4170537	up	Plxna4
6860163 93873	0.035458572	0.001231564	1.4162437	up	Pcdhb2
6974762 67207	0.010673828	8.86E-05	1.4154546	down	Lsm1
6899374 20200	0.044787455	0.001936602	1.4153138	up	S100a6
6950391 12576	0.048592288	0.002267077	1.414523	down	Cdkn1b
6934650 12909	0.015052847	1.75E-04	1.4131835	down	Crcp
6986031 11459	0.026061453	6.89E-04	1.4120103	down	Acta1
6847540 11820	0.006600066	4.53E-05	1.4108847	up	App
6965015 52432	0.01798404	2.89E-04	1.4097495	down	Ppp2r2d
6989473 319477	0.032513015	0.00101496	1.4095426	down	6030419C18Rik
6766368 26408	0.017905615	2.80E-04	1.4092246	up	Map3k5
6764056 66155	0.015204828	1.96E-04	1.4079518	down	Ufc1
6898502 213262	0.019556254	3.59E-04	1.4078732	up	Fstl5
6754403 11899	0.010969274	9.78E-05	1.4076041	up	Astn1
6938947 243043	0.008920094	7.09E-05	1.4064586	up	Kctd8
6838823 58200	0.006600066	4.59E-05	1.406405	down	Ppp1r1a
6813536 20745	0.04115921	0.001591286	1.405938	up	Spock1
6808773 13612	0.022202644	4.91E-04	1.4056443	up	Edil3
6915929,6915993 13131	0.015322137	2.04E-04	1.4053652	down	Dab1 Gm10304 2900034C19Rik AY512949 LOC100502604///Dab1
6817396 11534	0.024154648	5.98E-04	1.4021187	up	Adk
6993890 68743	0.01811096	2.98E-04	1.3999641	up	Anln
6995912 110119	0.015137184	1.91E-04	1.3997213	up	Mpi
6940592 246293	0.006600066	4.57E-05	1.3995645	down	Klhl8
6963534 320878	0.042509187	0.001677097	1.3995601	down	Mical2

6842682 17968	0.013328801	1.41E-04	1.3989094	up	Ncam2
6992332 14775	0.014431601	1.60E-04	1.3986729	down	Gpx1
6891689 241688	0.03991207	0.001487267	1.397334	up	6330439K17Rik
6888751 228355	0.018393353	3.12E-04	1.3969011	up	Madd
6891322 59030	0.01630344	2.38E-04	1.3968654	down	Mkks
6940431,6940432 72145	0.019232834	3.39E-04	1.3944072	up	Wdfy3
6852358,6925574 433759	0.0332504	0.001077326	1.3940427	up	Hdac1
6816124,6838415 22143	0.010677658	9.16E-05	1.3939478	up	Il31ra Tubal1b Gm5620 Tubal1b Gm6682 Gm5620
6838382 69612	0.044053618	0.00187886	1.3932033	down	2310037124Rik
6793649 50496	0.010673828	8.83E-05	1.3926133	down	E2f6
6896519 20482	0.019556254	3.70E-04	1.3922062	down	Skil
6918720 20810	0.024526443	6.18E-04	1.3916972	down	Srm
6760754 16560	0.021126166	4.38E-04	1.390481	up	Kif1a
6949797,6957119 14792	0.021159004	4.41E-04	1.38964	down	Lpcat3 Emg1 Lpcat3
6867701 56464	0.021438045	4.49E-04	1.3882275	up	Ctsf
6791418 15114	0.018393353	3.11E-04	1.3881177	up	Hap1
6918042 69116	0.01984823	3.94E-04	1.3880422	up	Ubr4 C230096C10Rik
6918042 230866	0.01984823	3.94E-04	1.3880422	up	Ubr4 C230096C10Rik
6803358,6803364 76559	0.016230881	2.34E-04	1.3870988	up	Atg2b
6958256 79362	0.011055893	1.00E-04	1.3868607	up	Bhlhe41
6785943,6978341 20021	0.01608881	2.27E-04	1.3867203	down	Polr2c
6793255,6804226 74682	0.015052847	1.76E-04	1.3839858	up	Wdr35 Wdr35 Matn3
6952137 320405	0.007270184	5.21E-05	1.3828329	up	Cadps2
6891454 75812	0.015441114	2.08E-04	1.3827794	down	Tasp1
6775098,6776193 67270	0.013542953	1.46E-04	1.3822339	down	Mrp42
6871837 271564	0.022187717	4.85E-04	1.3803174	up	Vps13a
6955205 66881	0.04115921	0.001587085	1.3796992	up	Pcyox1
6964023 28018	0.015322137	2.06E-04	1.3796805	down	Ubfd1
6949361 232337	0.043720026	0.001840079	1.3792504	down	Zfp637
6996440 235442	0.030360658	9.04E-04	1.3790128	up	Rab8b
6766110 15273	0.015052847	1.80E-04	1.3786916	down	Hivep2
6977075 66498	0.039544	0.001470771	1.3776422	down	Ddal
6992215 56808	0.049109604	0.002316849	1.3775046	up	Cacna2d2
6868032 54525	0.024762	6.32E-04	1.3772434	up	Syt7
6840923 268890	0.040423766	0.001535315	1.3750381	up	Lsamp
6971344 66422	0.04337046	0.001766213	1.3750355	down	Dctpp1
6885482 52838	0.022202644	4.92E-04	1.3748771	down	Dnlz
6767631 209462	0.034334507	0.001142994	1.3747562	down	Hace1
6964244 26417	0.01984823	3.89E-04	1.3736368	up	Mapk3
6968453 64176	0.008398302	6.26E-05	1.3733315	up	Sv2b
7017600 16728	0.019556254	3.56E-04	1.3732485	up	L1cam
6910621 68810	0.007590169	5.50E-05	1.3714011	down	Nexn
7008100 50918	0.019556254	3.65E-04	1.3707279	up	Myadm Prkcc
7008100 18752	0.019556254	3.65E-04	1.3707279	up	Myadm Prkcc
6820088 213484	0.04208484	0.001642205	1.3698381	down	Nudt18

6983927 66714	0.035799697	0.001245929	1.3697839	down	4921524J17Rik
6913020 230103	0.024526443	6.17E-04	1.3680389	up	Npr2
6963211 14356	0.043720026	0.001844338	1.3680122	down	Fxc1 Dnhd1 Gm9571
6963211 77505	0.043720026	0.001844338	1.3680122	down	Fxc1 Dnhd1 Gm9571
6963211 672646	0.043720026	0.001844338	1.3680122	down	Fxc1 Dnhd1 Gm9571
6799524 108089	0.01798404	2.92E-04	1.3673081	down	Rnf144a
6882521 66734	0.032802183	0.001035519	1.3671783	down	Map1lc3a
6971688 77938	0.0332504	0.001091005	1.3666912	down	Fam53b
6789401 104457	0.01297834	1.32E-04	1.3666172	down	0610010K14Rik
6899747,6899750, 6899752,6907246, 6907247 15077	0.024054471	5.86E-04	1.3645159	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1 Hist2h3c1 Hist2h3c2- ps Hist2h3b Hist2h3c1 Hist2h3c2- ps Hist2h3c1 Hist2h3c2- ps Hist2h3b Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6909139 109676	0.027723162	7.74E-04	1.3641738	up	Ank2 Gm4392
6909139 1000433 64	0.027723162	7.74E-04	1.3641738	up	Ank2 Gm4392
7017627 27643	0.039544	0.001465421	1.36293	down	Ubl4 Slc10a3-ubl4
6985851 18117	0.04531207	0.001965658	1.3628076	down	Cox4ub
6842933 74112	0.03459776	0.001165178	1.3626226	down	Usp16
6959133 66071	0.013328801	1.40E-04	1.3620924	up	Ethel
6780844,6788069  21408	0.034624055	0.001174939	1.3607397	down	Zfp354a Zfp354b 9230009I0 2Rik Zfp354b Zfp354a
6780844,6788069  27274	0.034624055	0.001174939	1.3607397	down	Zfp354a Zfp354b 9230009I0 2Rik Zfp354b Zfp354a
6750868 74205	0.0281969	8.07E-04	1.3601534	up	Acs13 Utp14b
6750868 195434	0.0281969	8.07E-04	1.3601534	up	Acs13 Utp14b
6755222 12847	0.019556254	3.53E-04	1.3600298	up	Copa
6812894 20238	0.009567102	7.67E-05	1.3594275	down	Atxn1
6775741 28088	0.01798404	2.93E-04	1.3590493	up	D10Wsu52e
6917217 242667	0.012750876	1.27E-04	1.3580503	down	Dlgap3
6778583 216527	0.01630344	2.39E-04	1.3575718	down	Ccm2
6912213 68493	0.022382699	5.02E-04	1.3574581	down	Ndufaf4
6855051 12268	0.026502775	7.17E-04	1.3574362	up	C4b C4a
6855051 625018	0.026502775	7.17E-04	1.3574362	up	C4b C4a
6805245,6811686  319187	0.030477278	9.11E-04	1.3565937	down	Hist1h2bn Hist1h2be Hist1h1e Hist1h2bn
6786991 75572	0.013328801	1.39E-04	1.3564721	down	Acyp2 Ccdc47
6935370 14086	0.036739744	0.001301056	1.3564117	down	Fscn1
6995661 330941	0.010969274	9.87E-05	1.3559855	down	AI593442
6939985 67111	0.038066395	0.001372992	1.3558711	up	Naaa
6998707 74443	0.037047874	0.001321823	1.3553175	up	P4htm
6947760 103963	0.028027382	7.95E-04	1.3548398	up	Rpn1
6825371 110265	0.033087827	0.001056168	1.3541646	down	Msra
6997077 71538	0.014639024	1.65E-04	1.3531889	down	Fbxo9
6785742 64660	0.016510215	2.46E-04	1.3525581	down	Mrps24
6880540 228550	0.008480565	6.52E-05	1.3519324	down	Itpka
6848513 68842	0.022382699	5.03E-04	1.3511229	up	Tulp4
6925345 66938	0.024154648	5.92E-04	1.350936	down	1700029G01Rik
6888720 66461	0.03315913	0.001060775	1.3498696	down	Ptpmt1

6978291 17748	0.018016174	2.95E-04	1.3498284	up	Mtl
7010345 236733	0.019556254	3.60E-04	1.3488789	up	Usp11
6754014 117198	0.00536721	3.51E-05	1.3487188	down	Ivns1abp
6935524 264064	0.016230881	2.35E-04	1.3464878	down	Cdk8
6929919 231148	0.010677658	9.22E-05	1.346334	down	Ablim2
6833138 22146	0.042509187	0.001688896	1.3447404	up	Tuba1c Gm6682 Gm8973
6833138 668092	0.042509187	0.001688896	1.3447404	up	Tuba1c Gm6682 Gm8973
6963264 60510	0.04670013	0.002075124	1.3440274	up	Syt9
6916797 29871	0.02366127	5.54E-04	1.3433441	down	Scmhl
6892193 68559	0.023056254	5.33E-04	1.3426592	down	Pdrg1
6941761 207565	0.04337046	0.001796049	1.3405432	down	Camkk2
6998396 20818	0.031038841	9.40E-04	1.340059	up	Srprb
6852902 17688	0.04679768	0.002089329	1.3396536	up	Msh6 Fbxo11
6852902 225055	0.04679768	0.002089329	1.3396536	up	Msh6 Fbxo11
6883127 57138	0.03864976	0.001417799	1.3395128	up	Slc12a5
6761155 27392	0.026663529	7.27E-04	1.339372	up	Pign
6788411 11927	0.011055893	1.01E-04	1.3388278	down	Atox1
6845459 207227	0.024154648	5.95E-04	1.3388058	up	Stxbp5l
6771920 270685	0.035340734	0.001215047	1.3384888	up	Mthfd1l
6966339 56188	0.043720026	0.001841995	1.3383098	up	Fxyd1
6864062 108013	0.020886658	4.27E-04	1.337718	up	Celf4
6945914 66797	0.015983123	2.21E-04	1.3368968	up	Cntnap2 Ccnl
6811806 22360	0.04193666	0.001624865	1.3367634	up	Nrsn1
6782456 19062	0.03324496	0.001070845	1.3359902	up	Inpp5k
6775310 70294	0.04337046	0.001783667	1.3358172	down	Rnf126
6840579 22042	0.03840255	0.001390516	1.3344265	down	Tfrc
6975876 192169	0.019556254	3.61E-04	1.3340727	down	Ufsp2
6754137 67792	0.017340807	2.65E-04	1.3336473	down	Rgs8
6917790 71665	0.03294127	0.001046858	1.3336054	up	Fuca1
6850421 17850	0.044442587	0.001907872	1.3334374	up	Mut Cenpq
6767258 14360	0.016510215	2.48E-04	1.3333771	down	Fyn
6908146 20912	0.04368416	0.001822909	1.3332828	up	Stxbp3a
6755173,6764068 21945	0.01910948	3.33E-04	1.3316907	down	Dedd//Nit1 Dedd
6896518 18759	0.010822849	9.44E-05	1.3315817	down	Prkci
7014815 110651	0.034624055	0.00116928	1.331539	down	Rps6ka3
6807437 75731	0.015854789	2.17E-04	1.3310698	down	5133401N09Rik
6883013 228858	0.0332504	0.001087326	1.3309959	up	Gdap1l1
6827203 72486	0.04312426	0.001746437	1.3300443	up	Rnf219
7010647 72693	0.043053027	0.001733175	1.3294554	up	Zcche12
6916125 230584	0.020587178	4.20E-04	1.3290225	up	Yipf1 Rfc5
6868899 22359	0.03324496	0.001071679	1.3275667	up	Vldlr
6966328 22282	0.01394069	1.53E-04	1.3273046	down	Usf2
6929719 14284	0.027805798	7.85E-04	1.326915	down	Fosl2
6992328 66257	0.042509187	0.00168081	1.3260579	up	Nicn1
6831592 22701	0.04244813	0.00166235	1.3257983	down	Zfp41
6869635,6873083 67590	0.01798404	2.92E-04	1.3250004	down	Entpd1 Tctn3//Tctn3
6749455 227095	0.038871896	0.001429375	1.3242575	up	Hibch
6896593 67414	0.04199467	0.001632782	1.3226247	up	Mfnl
6818742 93834	0.011135913	1.05E-04	1.3224422	down	Peli2
6993465 71946	0.04337046	0.001770237	1.320743	up	Endod1
6884352 50497	0.034411497	0.001156486	1.3202697	down	Hspa14



6874080 73442	0.025432337	6.54E-04	1.3201097	up	Hspa12a
6931961 319387	0.023703147	5.68E-04	1.3191973	up	Lphn3 Dynl1a A230055J12Rik
6931961 320314	0.023703147	5.68E-04	1.3191973	up	Lphn3 Dynl1a A230055J12Rik
6845559 76916	0.043053027	0.001732094	1.3186158	down	4930455C21Rik
6937073 14208	0.032301586	9.90E-04	1.3180437	up	Ppm1g
6759718 21961	0.022454733	5.11E-04	1.3180168	down	Tnsl
6869973 226151	0.032512043	0.001010358	1.3166649	up	Fam178a
6787293 23964	0.043053027	0.001730065	1.3164718	up	Odz2
6757896 320011	0.04584206	0.00202088	1.3162661	up	Uggt1
6933812 57816	0.016510215	2.45E-04	1.3148854	down	Tesc
6878657 241520	0.043053027	0.001734471	1.3144302	up	Fam171b
6884183 72075	0.026050128	6.84E-04	1.3144196	down	Ogfr
6935927 13121	0.02441559	6.10E-04	1.314148	up	Cyp51
6833185 14555	0.034411497	0.001153731	1.3139409	down	Gpd1
6792129 217265	0.015052847	1.82E-04	1.3136501	up	Abca5
6757120 29819	0.023703147	5.65E-04	1.3135145	down	Stau2 C130013N14Rik
6757120 402742	0.023703147	5.65E-04	1.3135145	down	Stau2 C130013N14Rik
6789979 69713	0.026313707	7.05E-04	1.3133277	down	Nlk Pin4
6776152 67723	0.022454733	5.12E-04	1.3133212	up	4932415G12Rik
6857310 72722	0.017905615	2.82E-04	1.3130908	down	Fam98a
6966588 19777	0.02366127	5.57E-04	1.3123834	down	C80913
6774684 211488	0.02650006	7.12E-04	1.3117256	down	Ado
6768323 73132	0.042509187	0.00169164	1.3114651	down	Slc25a16
6840019 75826	0.022007378	4.78E-04	1.3112297	down	Senp2
6964259 233878	0.01566359	2.13E-04	1.3110644	up	Sez6l2
6892364 228812	0.019560797	3.74E-04	1.3108152	up	Pigu
6832719 12805	0.043834306	0.001859543	1.3107749	up	Cntn1
6768094 19386	0.023703147	5.62E-04	1.3087014	down	Ranbp2
6873254 73689	0.019560797	3.77E-04	1.3083574	down	Bloc1s2
6902661 12972	0.019008702	3.27E-04	1.3077829	up	Cryz
6974039 54126	0.022860363	5.27E-04	1.3058306	down	Arhgef7
6896584,6904047 22401	0.015137184	1.90E-04	1.305752	down	4930429B21Rik Zmat3 Zmat3
6966187 73833	0.024154648	5.94E-04	1.3053551	down	Rasgrp4 Fam98c
6797707 73046	0.04815002	0.00224111	1.3049716	down	Glx5
6918705 230904	0.03637223	0.001270969	1.3044555	up	Fbxo2
6988773 22687	0.03992887	0.001493508	1.3041425	down	Zfp259
6969028 14085	0.049958326	0.002381109	1.3041215	up	Fah
6810280 268706	0.043053027	0.001727578	1.3038671	up	Slc38a9
6853762 26407	0.014973253	1.72E-04	1.3027297	up	Map3k4
6789979,6888496 18099	0.029707763	8.73E-04	1.3026756	down	Nlk Pin4 Olf1111 Nlk
6763652 98376	0.048653852	0.002278244	1.3022286	up	Gorab
7017627,7017628 100169864	0.036739744	0.001300395	1.3021116	down	Ubl4 Slc10a3-ubi4 Slc10a3 Slc10a3-ubi4
6831994 11911	0.022598844	5.16E-04	1.3008779	down	Atf4
6770325 103098	0.029003233	8.40E-04	1.3007712	up	Slc6a15
6876173 227723	0.018353892	3.07E-04	1.299692	up	Bat2l
6864678 67199	0.034411497	0.001152159	1.2993454	down	Pfdn1
6881771 18549	0.03294127	0.001044944	1.2993256	up	Pesk2
6823041,6823100,	0.047668647	0.002167039	1.2989156	up	Camk2g Usp54 Usp54

6823105 78787						
6884721 50755	0.012751671	1.28E-04	1.2987964	down	Fbxo18	
6917489 66464	0.0358883	0.001251536	1.2987165	down	Taf12	
6966164 24030	0.019755332	3.85E-04	1.2983397	down	Mrps12	
6877931 73373	0.043834306	0.001858988	1.2981822	down	Phospho2 Rbm3	
6788020 12330	0.021695498	4.64E-04	1.2979654	up	Canx	
6955766 101351	0.035250623	0.001209471	1.2979203	up	A130022J15Rik	
6823710 64652	0.019556254	3.64E-04	1.2974981	up	Nisch	
6966600 12447	0.021552088	4.59E-04	1.2974267	up	Ccne1	
6954572 104263	0.045719497	0.002007459	1.2972401	up	Kdm3a	
6958995 403187	0.027723162	7.76E-04	1.296651	down	Opa3	
6899585 78523	0.039544	0.001469816	1.2958944	down	Mrpl9	
6782088,6789369 54351	0.036654945	0.00128858	1.2950375	down	Dullard Rai12///Rai12	
6944432 76522	0.046211697	0.002040424	1.2948897	down	Naa38	
6915745 242557	0.047974896	0.002199229	1.2935965	down	Atg4c Gm12689 Gm10305	
6915745 100137011	0.047974896	0.002199229	1.2935965	down	Atg4c Gm12689 Gm10305	
6915745 100038722	0.047974896	0.002199229	1.2935965	down	Atg4c Gm12689 Gm10305	
6840527 66994	0.022202644	4.91E-04	1.2931771	down	I500031L02Rik	
6867642 66990	0.040423766	0.001546125	1.2931631	down	Tmem134	
6949153 232333	0.03481309	0.001184668	1.2929022	up	Slc6a1	
6945614,6952941 54484	0.039544	0.001463646	1.2925439	down	Mkrn1	
6783654 71452	0.019560797	3.78E-04	1.2917719	down	Ankrd40	
6791541 268490	0.024805788	6.35E-04	1.2917227	down	Lsm12	
6929125 330050	0.044053618	0.001880092	1.2914281	up	Fam185a	
6864695 24068	0.018772775	3.22E-04	1.2907568	down	Sral	
6769213,7008703 216166	0.04026438	0.001520212	1.290465	up	Plk5///Plk5 Spt1 LOC236598	
6899743 64051	0.02606873	6.94E-04	1.2904557	up	Sv2a	
6775372 66594	0.03663139	0.001285176	1.2897568	down	Uqcr11	
6929457,6929458 13483	0.04954692	0.002347931	1.289421	up	Dpp6	
6977142 17274	0.04013421	0.00150401	1.289129	down	Rab8a	
6958407 387314	0.031038841	9.38E-04	1.2888066	up	Tmtcl	
6936116 23857	0.044442587	0.001903648	1.2882978	down	Dmtfl	
6899613 229584	0.031288665	9.55E-04	1.2878227	up	Pogz	
6962925 70974	0.022454733	5.08E-04	1.2877574	up	Pgm2l1 Gpx2-ps1	
6938710 68552	0.024872728	6.38E-04	1.2877141	down	I110003E01Rik	
6836237 13196	0.04337046	0.00179329	1.2868526	down	Asap1 9130004J05Rik Gm10926 LOC100039024	
6836237 71603	0.04337046	0.00179329	1.2868526	down	Asap1 9130004J05Rik Gm10926 LOC100039024	
6836237 100169872	0.04337046	0.00179329	1.2868526	down	Asap1 9130004J05Rik Gm10926 LOC100039024	
6836237 100039024	0.04337046	0.00179329	1.2868526	down	Asap1 9130004J05Rik Gm10926 LOC100039024	
6996269 26395	0.030709505	9.20E-04	1.2838286	up	Map2k1	
6842326 19876	0.04390929	0.001867758	1.2838104	up	Robo1	
6833186 66379	0.044442587	0.001914988	1.28336	down	2310016M24Rik	
6924281 56280	0.04115921	0.001585019	1.2821487	down	Mrpl37	

6852767 19043	0.021552088	4.58E-04	1.2815548	down	Ppm1b
6788141 76901	0.016510215	2.48E-04	1.2815293	up	Phf15
6952900 15258	0.04584206	0.002018127	1.2813956	up	Hipk2
6975050 66959	0.04953374	0.002343824	1.2809025	down	Dusp26
6755233 140559	0.021552088	4.59E-04	1.2806572	up	Igsf8
6765307 214791	0.019556254	3.64E-04	1.2801203	down	Sertad4
6780767 14584	0.04337046	0.001787895	1.2799969	up	Gfpt2
6962930 320452	0.046510797	0.00206017	1.2792466	up	P4ha3
6750149 66646	0.019556254	3.50E-04	1.2789862	down	Rpe
6801914,6962925 14776	0.02474206	6.30E-04	1.2780323	up	Gpx2 Gpx2-ps1 Pgm21 Gpx2-ps1
6801914,6962925 14777	0.02474206	6.30E-04	1.2780323	up	Gpx2 Gpx2-ps1 Pgm21 Gpx2-ps1
6991027 21983	0.04880326	0.002292103	1.2778425	up	Tpbp
6816317 52552	0.026050128	6.85E-04	1.2773947	down	Parp8
6895393 11308	0.029042374	8.43E-04	1.2773659	down	Abi1
6970568 68815	0.019560797	3.78E-04	1.2766405	down	Btbd10
6768897 103172	0.03806482	0.001366233	1.2742038	down	Chchd10
6793253,6804226 17182	0.042109743	0.001646138	1.2741894	up	Matn3 Wdr35 Matn3
6820237 67381	0.016230881	2.33E-04	1.2731138	down	Med4
6992367 19087	0.047316674	0.002136069	1.2726023	up	Prkar2a
6754205,7011852 15354	0.024154648	5.97E-04	1.2718637	down	Stx6 Hmgb3 Hmgb3
6989440 13070	0.047316674	0.002129709	1.2717532	down	Cyp11a1
6819928 239157	0.03260038	0.001023769	1.2716821	up	Pnma2
6964329 68961	0.027616503	7.65E-04	1.2709464	down	Phkg2 Gml66
6964329 233899	0.027616503	7.65E-04	1.2709464	down	Phkg2 Gml66
6896770 229211	0.04261202	0.001698729	1.2707958	up	Acad9
6819694,6825302 13030	0.015052847	1.86E-04	1.2706757	up	Ctsb Fdf1 Fdf1 Ctsb
6819694,6825302 14137	0.015052847	1.86E-04	1.2706757	up	Ctsb Fdf1 Fdf1 Ctsb
6980270 13642	0.021932513	4.73E-04	1.2703769	up	Efnb2
6824779 59049	0.0354481	0.001228634	1.2695707	up	Slc22a17
6922895,6922901 69863	0.038421385	0.001402961	1.2689745	down	Ttc39b
6942675 100494	0.031003293	9.31E-04	1.268897	down	Zfand2a
6854541 56409	0.026039083	6.79E-04	1.2682208	down	Nudt3 Anks1
6837470 29859	0.042509187	0.001683426	1.2679896	down	Sult4a1
6881337 12653	0.044442587	0.00191544	1.2677501	up	Chgb
6823721 24056	0.032360055	9.94E-04	1.2668406	up	Sh3bp5 Capn7
6759905 13838	0.033567186	0.00110687	1.2665352	up	Epha4
6875602 74159	0.0469654	0.002106723	1.2665263	down	Acbd5
6822891 218772	0.02661468	7.22E-04	1.2661253	down	Rarb Rpl23a
6791233 12295	0.017905615	2.82E-04	1.2653434	down	Caenb1
6764662 226757	0.032434884	0.001001119	1.2649517	down	Wdr26
6937844 16826	0.03260038	0.00102078	1.2648244	up	Ldb2
6754526 73844	0.036409926	0.001274847	1.264413	up	Ankrd45
6834745 223455	0.040423766	0.001534566	1.2642958	up	6-Mar
6792787 209011	0.02366127	5.58E-04	1.2642294	down	Sirt7
6837189 66538	0.03840255	0.001389966	1.2636565	down	Rps19bp1
6769192 66043	0.02366127	5.59E-04	1.263041	down	Atp5d

6781561 72795	0.047668647	0.002171312	1.2627603	down	Ttc19
6785665 66152	0.040423766	0.00154082	1.2617928	down	Uqcr10
6980964 18970	0.03806482	0.001370259	1.2614816	down	Polb A930013F10Rik
6980964 68074	0.03806482	0.001370259	1.2614816	down	Polb A930013F10Rik
6760006 69368	0.043720026	0.001832152	1.2613966	up	Wdfy1
6754437,6957465, 7011663,7018291  26374	0.027663546	7.68E-04	1.2612764	down	Rfwd2 Scarna3a Csd Rfwd 2 Ctag2 Rfwd2 Asb12 Rfw d2
6784785 13929	0.026686419	7.31E-04	1.2611614	down	Amz2
6883098 52840	0.027805798	7.82E-04	1.2610734	down	Dbndd2
7014836 58194	0.025439167	6.56E-04	1.2605152	down	Sh3kbp1 Map3k15
6757634,6873078, 6875459 19243	0.024367737	6.06E-04	1.260183	down	Ptp4a1 Gm13363 Gm13363  Ptp4a1 Etl4 Gm13363 Ptp4a 1 Gm16495
6757634,6873078, 6875459 433406	0.024367737	6.06E-04	1.260183	down	Ptp4a1 Gm13363 Gm13363  Ptp4a1 Etl4 Gm13363 Ptp4a 1 Gm16495
6815255 66549	0.032440964	0.001005171	1.260047	down	Aggf1
6861350 12322	0.03260038	0.001024564	1.2599939	up	Camk2a
6833138,6838415, 6838417 626534	0.0332504	0.001091748	1.2594355	up	Tuba1c Gm6682 Gm8973 T uba1b Gm6682 Gm5620 Tu ba1a Gm6682 Gm5620
6987128 69137	0.026650216	7.25E-04	1.2593307	up	2200002K05Rik
6942276 212996	0.038421385	0.001404705	1.2593135	down	Wbscr17
6972990 22192	0.0332504	0.001091686	1.2590938	down	Ube2m
6901732 108943	0.033052154	0.001052705	1.2589556	down	Rg9mtd2
6782708 55978	0.02366127	5.52E-04	1.2587875	down	Ift20
6985355 20340	0.045719497	0.002009048	1.2586819	up	Glg1
7009774 20977	0.032272834	9.87E-04	1.2579204	up	Syp
6788993 70383	0.034411497	0.001154944	1.2574023	down	Cox10
6835104 54375	0.01984823	3.91E-04	1.2570033	down	Azin1
6798218 17169	0.02474206	6.29E-04	1.2569531	down	Mark3
6817229,6822949  69721	0.040423766	0.001545631	1.255124	down	Nkiras1 Ube2e1 Nkiras1
6985984 78779	0.040423766	0.001540842	1.2541198	down	Spata2L
6966425 14751	0.040252663	0.001513779	1.2531556	up	Gpi1
6949084 68089	0.031038841	9.36E-04	1.2528758	down	Arpc4
6814385 18570	0.04533531	0.001976228	1.2527531	down	Pded6
6926505 71529	0.036739744	0.001301894	1.2519644	down	9030409G11Rik
6994589 109229	0.03402511	0.001129147	1.2509396	down	Fam118b Srpr
6886244,6894961  94217	0.027616503	7.61E-04	1.2506616	up	Lrp1b Ran Lrp1b 4631405J 19Rik
6825888 16432	0.0354481	0.001228708	1.2497038	up	Itm2b
6806831 218215	0.04793611	0.00218734	1.2494488	up	Rnf144b
6849525,6854541  224650	0.03324496	0.001072871	1.2488078	down	Anks1 Nudt3 Anks1
6908149 66921	0.034624055	0.001175801	1.2484398	up	Prpf38b
6860133 70791	0.04873302	0.002285377	1.2480017	down	Hars2
6850552 83965	0.04799745	0.002207012	1.247138	up	Enpp5
6839957 78408	0.026502775	7.14E-04	1.2471005	down	Fam131a
6847324,6850940  12228	0.04811016	0.002220749	1.2462183	down	Btg3 Gm7334
6847324,6850940  654432	0.04811016	0.002220749	1.2462183	down	Btg3 Gm7334

6943067 74132	0.032513015	0.001013204	1.2455171	down	Rnf6
6805241,6805355 69386	0.04533531	0.001976078	1.245417	down	Hist1h4b Hist1h4j Hist1h4k Gm11275 Hist1h4b
6816124,6838415,6838417 434428	0.038419306	0.001399227	1.2448874	up	Il31ra Tuba1b Gm5620 Tuba1b Gm6682 Gm5620 Tuba1a Gm6682 Gm5620
6970442 67150	0.048592288	0.002268528	1.2442167	down	Rnf141
6805252,6805385,6811537,6811564,6811678,6811692,6811701 319157	0.03864976	0.001418489	1.2436203	down	Gm11275 Hist1h4a Hist1h4b Hist1h4f Hist1h4i Hist1h4m Hist1h4a Hist1h4b Hist1h4c Hist1h4f Hist1h4m Hist1h4i Hist1h4f Hist1h4m Gm11275 Hist1h4a Hist1h4b Hist1h4c Hist1h4f Hist1h4m Gm11275 Hist1h4a Hist1h4c Hist1h4f Hist1h4a Hist1h4b Hist1h4f Hist1h4m
6898063 16497	0.027723162	7.78E-04	1.2418925	down	Kenab1
6903454 13123	0.043608375	0.001815099	1.2418736	up	Cyp7b1
6867650 19045	0.023736937	5.72E-04	1.2415985	down	Ppp1ca
6965901 232975	0.026686419	7.32E-04	1.241252	up	Atp1a3
6968647 67308	0.04337046	0.001792819	1.2392359	down	Mrpl46
6942655 19085	0.04811016	0.002225725	1.2391682	up	Prkar1b 9330169B04Rik
6942655 319999	0.04811016	0.002225725	1.2391682	up	Prkar1b 9330169B04Rik
6782454 18738	0.04368416	0.001824396	1.2384719	down	Pitpna
6883526 109054	0.042509187	0.001680835	1.2382039	down	Pfdn4 Cyp24a1
6919195 140500	0.036743402	0.001304607	1.2380875	down	Acap3
6777305 64050	0.02606873	6.95E-04	1.2364174	down	Yeats4
6819425 67840	0.03992887	0.001491343	1.2361919	down	Mrp63
6805252,6805385,6811528,6811537,6811678,6811692,6811701 326619	0.043608375	0.001812688	1.2355359	down	Gm11275 Hist1h4a Hist1h4b Hist1h4f Hist1h4j Hist1h4m Hist1h4a Hist1h4b Hist1h4c Hist1h4f Hist1h4m Hist1h4a Hist1h4b Hist1h4j Hist1h4k Hist1h4m Hist1h4a Hist1h4b Hist1h4c Hist1h4f Hist1h4m Gm11275 Hist1h4a Hist1h4c Hist1h4f Hist1h4a Hist1h4b Hist1h4f Hist1h4m
6975209 75029	0.035077687	0.001196139	1.2351745	down	Purg
6805252,6811537,6811564 319158	0.035202216	0.001205335	1.2343416	down	Gm11275 Hist1h4a Hist1h4b Hist1h4f Hist1h4i Hist1h4m Hist1h4i Hist1h4f Hist1h4m Gm11275
6973683 140482	0.04048394	0.001551273	1.234045	up	Zfp358
6998583 109652	0.043834306	0.001861487	1.2332873	down	Acyl
6867748 69860	0.0393026	0.001447976	1.2314234	down	Eif1ad Sart1
6864330 67453	0.046591923	0.00206704	1.2267478	down	Slc25a46
6849973 66416	0.030015303	8.88E-04	1.2261399	down	Ndufa7
6837428 109754	0.049655594	0.002356572	1.2259744	up	Cyb5r3
6767460 54198	0.0332504	0.001090932	1.2253067	down	Snx3
6789483 103712	0.044442587	0.001914789	1.2245939	up	6330403K07Rik
6876310 227743	0.047316674	0.002145768	1.2222756	down	Mapkap1 5830434F19Rik 4930414H07Rik

6876310 76034	0.047316674	0.002145768	1.2222756	down	Mapkap1 5830434F19Rik 4930414H07Rik
6876310 73869	0.047316674	0.002145768	1.2222756	down	Mapkap1 5830434F19Rik 4930414H07Rik
6860049 56550	0.04629016	0.002047143	1.222038	down	Ubc2d2
6917283 107271	0.042509187	0.001680853	1.2182815	down	Yars
6951756 101148	0.04815002	0.002235392	1.2171097	down	B630005N14Rik
7012681 17698	0.049823217	0.00236803	1.2154173	up	Msn
6833184 83797	0.04026438	0.001519246	1.2153908	down	Smared1
6839932 11773	0.035368353	0.00122097	1.2150815	down	Ap2m1
6762234 21367	0.045408387	0.001985799	1.2126511	up	Cntn2
6853197 76781	0.04337046	0.001784526	1.210731	down	Mettl4
6764138 98660	0.04533531	0.00197496	1.2105742	up	Atp1a2
6794073 380752	0.045719497	0.002007306	1.2088413	down	Tssc1
6965153 330671	0.043053027	0.001731272	1.2070053	up	B4galnt4
6749572 19070	0.046761967	0.002081159	1.2048521	down	Mobkl3
6947596 21802	0.0469654	0.0021037	1.2038522	down	Tgfa

[00136] Table 3. Genes with significant changes (Benjamini-Hochberg adjusted p-values < 0.05) of at least 1.2-fold up or down in Drd1a-expressing cortical neurons at 2 years and 6 weeks of age, as compared to 6 weeks of age.

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene symbol
7023132 236604	1.30E-04	1.92E-08	3.5778549	up	Pisd-ps3 Pisd-ps1
6845079 11815	0.047086563	2.74E-04	2.9835136	up	Apod
6998397 22041	0.002609346	1.33E-06	2.6085126	up	Trf
6972168 66141	0.002609346	1.85E-06	2.5289943	up	Ifitm3
6937190,7023132 320951	1.30E-04	2.74E-08	2.4953797	up	Pisd Pisd-ps3///Pisd-ps3 Pisd-ps1
6937190,7023132 66776	1.30E-04	2.74E-08	2.4953797	up	Pisd Pisd-ps3///Pisd-ps3 Pisd-ps1
6791465 19183	0.03733478	1.49E-04	2.4761345	up	Psmc3ip
6784526 17896	0.032506455	6.81E-05	2.2456028	down	Myl4 Lin52 Gm7020
6817978 21924	0.032506455	6.86E-05	2.1837645	down	Tnnc1
6823429 66039	0.03972272	1.65E-04	2.117844	down	D14Erd449e
6784526,6796606 217708	0.028130708	5.04E-05	2.056382	down	Myl4 Lin52 Gm7020///Lin52 Gm7020
6784526,6796606 629959	0.028130708	5.04E-05	2.056382	down	Myl4 Lin52 Gm7020///Lin52 Gm7020
6862827 12405	0.047086563	2.66E-04	2.0361567	down	Cbln2
6899683 13040	0.003809435	3.48E-06	1.9884881	up	Ctss
6782484 74230	0.004386073	4.63E-06	1.9319992	down	1700016K19Rik
6959584 22177	0.040753897	1.83E-04	1.9228017	up	Tyrobp
6753402 21956	0.004028092	3.96E-06	1.8955778	up	Tnnt2
6983999 12404	0.047086563	2.98E-04	1.8022048	down	Cbln1
6869068 77125	0.028130708	5.05E-05	1.7979872	up	H33
6995918 235416	0.002609346	1.30E-06	1.7442707	down	Lman1 Cplx3
6995918 235415	0.002609346	1.30E-06	1.7442707	down	Lman1 Cplx3
6768261,6876138 432466	0.002609346	2.00E-06	1.6745123	up	Gm5424 Ass1///Ass1 Gm5424
6768261,6876138 11898	0.002609346	2.00E-06	1.6745123	up	Gm5424 Ass1///Ass1 Gm5424
6988976 13489	0.03615578	1.37E-04	1.6427418	up	Drd2
6957352 232400	0.040753897	1.83E-04	1.6084235	down	BC048546
6967593 110886	0.042847566	2.11E-04	1.5844014	down	Gabra5
6945335 109624	0.028130708	5.14E-05	1.5766602	up	Cald1

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene symbol
6747478 76982	0.035836473	1.04E-04	1.5673733	down	3110035E14Rik
6992215 56808	0.035454802	9.40E-05	1.5502318	up	Cacna2d2
6993890 68743	0.005008051	5.63E-06	1.5448154	up	Anln
6900928 66789	0.019637536	2.35E-05	1.5300947	down	Alg14
7016409 245386	0.03400331	7.41E-05	1.5258399	up	Fam70a Zbtb33
6954385 13197	0.035454802	8.96E-05	1.5098312	down	Gadd45a Gng12
6864456 27528	0.040753897	1.77E-04	1.5033208	down	D0H4S114
6836358 17988	0.028130708	4.14E-05	1.4998	up	Ndrp1
6811068 56048	0.035836473	1.21E-04	1.4984856	up	Lgals8
6869570 74055	0.047086563	2.79E-04	1.4937183	up	Pfcl1
6883533 76829	0.040753897	1.79E-04	1.4935141	down	Dok5
6872916 15925	0.028428873	5.41E-05	1.4907689	down	Ide
6764721 12334	0.03733478	1.48E-04	1.4689611	up	Capn2
6769343,6773537,6968533 624784	0.002609346	2.02E-06	1.4675822	down	Tdgl Gm9855 Gm5806
6769343,6773537,6968533 545124	0.002609346	2.02E-06	1.4675822	down	Tdgl Gm9855 Gm5806
6748020 14859	0.047086563	3.00E-04	1.4651384	up	Gsta3
6752222 241201	0.03615578	1.34E-04	1.4480729	up	Cdh7
7010762,7016409 56805	0.035454802	9.28E-05	1.4420869	up	Zbtb33 Fam70a Zbtb33
6946785,6954385 14701	0.035454802	9.47E-05	1.4353529	down	Gng12 Gadd45a Gng12
6769343,6773537,6775518,6968533 21665	0.0037269	3.14E-06	1.4327823	down	Tdgl Gm9855 Gm5806 Glt8d2 Tdg
6783321 18952	0.035836473	1.13E-04	1.4307998	up	Sept4 LOC100503535
6783321 100503535	0.035836473	1.13E-04	1.4307998	up	Sept4 LOC100503535
6758223 66297	0.043042764	2.19E-04	1.4281554	down	2610017J09Rik
6755559 68226	0.043042764	2.24E-04	1.4218508	down	Efcab2
6822729 54713	0.040753897	1.79E-04	1.4151258	down	Fezf2
6886678 74194	0.043677434	2.33E-04	1.4130437	down	Rnd3
6766409 52906	0.035454802	9.42E-05	1.4032575	up	Ahil1
6782702 22370	0.035836473	1.27E-04	1.4028069	up	Vtn
6913901 72479	0.035454802	8.00E-05	1.3892306	up	Hsd12
6909375 66357	0.02716284	3.63E-05	1.3778921	down	Oste
6923525 74519	0.028428873	5.60E-05	1.3728458	up	Cyp2j9
6912245,6920276 14348	0.03615578	1.37E-04	1.3615227	down	Fut9
6931790 57357	0.047086563	2.69E-04	1.359849	down	Srd5a3
6872646 54447	0.035836473	1.12E-04	1.3592392	up	Asah2
6862102 52538	0.03733478	1.52E-04	1.3579103	up	Acaa2
6959459 51798	0.03733478	1.52E-04	1.355676	up	Ech1
6816413 18115	0.024438534	3.09E-05	1.3556129	up	Nut
6995076 71732	0.04191086	2.03E-04	1.3523128	up	Vps11
6977260 15368	0.04191086	2.01E-04	1.343893	up	Hmox1
6959265 13086	0.049245864	3.25E-04	1.3420637	up	Cyp2a4 Cyp2a5
6959265 13087	0.049245864	3.25E-04	1.3420637	up	Cyp2a4 Cyp2a5
6796053 238266	0.028130708	4.59E-05	1.3297588	down	Syt16
6823849 26419	0.035836473	1.29E-04	1.3211268	down	Mapk8
6789475 216877	0.028130708	4.66E-05	1.3187447	up	Dhx33
6767387 53599	0.04191086	2.02E-04	1.3120232	down	Cd164
6974490 52123	0.043677434	2.33E-04	1.3106182	down	Agpat5
6761964 72160	0.047086563	2.94E-04	1.2954209	down	Tmem163 Mgat5
6853910 72057	0.035836473	1.25E-04	1.294048	down	Phf10 1600012H06Rik LOC106740
6853910 106740	0.035836473	1.25E-04	1.294048	down	Phf10 1600012H06Rik LOC106740
6750547 227292	0.041231222	1.91E-04	1.2933345	up	Ctdsp1
6864326 19762	0.035454802	9.20E-05	1.2931432	up	Rit2
6836298,6849523 20630	0.03548006	9.73E-05	1.2924098	down	Surpc

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene symbol
6924832 12795	0.035836473	1.25E-04	1.283983	down	Plk3
6922649 66928	0.047086563	2.68E-04	1.2812592	down	3110001D03Rik LOC280487
6922649 280487	0.047086563	2.68E-04	1.2812592	down	3110001D03Rik LOC280487
6825445 19229	0.043042764	2.19E-04	1.2802857	up	Plk2b
6941685 11669	0.047086563	2.91E-04	1.2797663	up	Aldh2
6991261 19417	0.035836473	1.28E-04	1.2791666	up	Rasgrf1
6998987 74100	0.047086563	2.94E-04	1.2663616	down	Arpp21 Mir128-2
6998987 723815	0.047086563	2.94E-04	1.2663616	down	Arpp21 Mir128-2
6925587 66264	0.047666077	3.08E-04	1.2616228	down	Ccdc28b 2510006D16Rik
6760754 16560	0.035836473	1.01E-04	1.2583791	up	Kif1a
6791015 18604	0.040834192	1.87E-04	1.2575148	up	Pdk2
6783029 70439	0.047086563	3.01E-04	1.2506527	down	Taf15
6988962 26951	0.047086563	2.80E-04	1.2481672	up	Zw10
6848806,6853910 67912	0.043042764	2.21E-04	1.2481549	down	1600012H06Rik Phf10 1600012H06Rik LOC106740
7010345 236733	0.047086563	2.90E-04	1.2121428	up	Usp11

[00137] Table 4. Genes with significant changes (Benjamini-Hochberg adjusted p-values < 0.05) of at least 1.2-fold up or down in Drd2-expressing cortical neurons at 2 years and 6 weeks of age, as compared to 6 weeks of age.

Gene_ID	p value (corrected)	p value	Fold change (absolute)	Regulation	Gene symbol
6998397 22041	0.015955767	1.91E-05	4.5832944	up	Trf
6813284 13488	0.008502543	5.38E-06	3.3188136	up	Drd1a
6845079 11815	0.010140898	8.28E-06	2.5178335	up	Apod
7023132 236604	0.003949368	5.46E-07	2.387355	up	Pisd-ps3 Pisd-ps1
6776577 67405	0.003949368	1.20E-06	2.2947934	down	Nts
6817978 21924	0.04098089	2.38E-04	2.2747989	down	Tnnc1
6754149,6861135 14645	0.01982543	3.35E-05	2.2733164	up	Glul Grand3 Glul
6877356,6886947 77767	0.032421894	1.41E-04	2.2179747	up	Gaint5 Ernn Ernn
6908075,6908077,6908078 4864	0.048785735	4.86E-04	2.0753336	up	Gstm6 Gstm3 Gstm3 Gstm1 Gstm3
6791494 73635	0.030455668	1.11E-04	2.0665221	down	Rundc1 1700113I22Rik Aarsd1
6937190,7023132 320951	0.003949368	1.39E-06	2.0144777	up	Pisd Pisd-ps3 Pisd-ps3 Pisd-ps1
6937190,7023132 66776	0.003949368	1.39E-06	2.0144777	up	Pisd Pisd-ps3 Pisd-ps3 Pisd-ps1
6943974 21333	0.030455668	1.07E-04	1.9952383	up	Tac1
7013389 237010	0.042421777	3.34E-04	1.8928168	up	Klhl4
6993890 68743	0.032484267	1.46E-04	1.884146	up	Anln
6898477 20713	0.03124751	1.34E-04	1.8833878	up	Serpini1
6880670 12010	0.041485418	2.56E-04	1.875716	up	B2m
6944262 114142	0.003949368	6.80E-07	1.8566908	up	Foxp2
6748020 14859	0.032484267	1.45E-04	1.8344905	up	Gsta3
6811068 56048	0.042421777	3.38E-04	1.8010027	up	Lgals8
6997555 382090	0.01285925	1.36E-05	1.7201661	up	4922501C03Rik
6904297 11747	0.030455668	1.20E-04	1.6902814	up	Anxa5
6862062 71263	0.043850936	3.92E-04	1.671545	down	Mro
6838811,6917301 17357	0.042421777	3.05E-04	1.6639088	down	Marcks1 BC048502 Marcks1l
6964527 56213	0.045647837	4.30E-04	1.6607143	up	Hra1
6788025 216724	0.022754725	5.07E-05	1.6575161	up	Rufy1
6973587 11816	0.010140898	9.98E-06	1.6567526	up	Apoe



6899520 20194	0.03124751	1.33E-04	1.6538708	up	S100a10
6878655 16410	0.039288376	2.13E-04	1.64845	up	Ilgav
6834890 56274	0.042421777	2.95E-04	1.6482366	up	Stk3
6836991 12300	0.030455668	1.19E-04	1.6434618	down	Cacng2
6989222 12903	0.008459655	4.76E-06	1.639584	down	Crabp1
6971344 66422	0.0259438	6.93E-05	1.6349066	down	Dcrrp1
6884986 74103	0.046546645	4.49E-04	1.6318291	down	Nebi
6797969 17263	0.022754725	5.76E-05	1.6306723	down	Meg3 Dlk1 Mir1906
6797969 100316809	0.022754725	5.76E-05	1.6306723	down	Meg3 Dlk1 Mir1906
6764138 98660	0.048785735	4.85E-04	1.6294948	up	Atp1a2
6899747,6907247 15267	0.010140898	9.80E-06	1.6212646	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1 Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6899747,6907247 319192	0.010140898	9.80E-06	1.6212646	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1 Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6961010 17984	0.00482493	2.04E-06	1.6177676	up	Ndn
6926936 110208	0.042801354	3.71E-04	1.6117427	up	Pgd
6861751 52662	0.033511773	1.53E-04	1.6040033	down	D18Ertid653e
6823068 11750	0.016177624	2.05E-05	1.5961775	up	Anxa7
6913009,6921154 12517	0.042801354	3.69E-04	1.5803419	down	Tesk1 Cd72 Cd72
6885395 68475	0.02753681	7.94E-05	1.5702732	down	Ssnal
6972710 57776	0.04098089	2.43E-04	1.5661737	down	Ttyh1
7000764 77226	0.030455668	1.24E-04	1.565689	down	9330169L03Rik
6961650,6968387 100038347	0.017847234	2.71E-05	1.564204	down	Fam174b
6753402 21956	0.00988544	6.95E-06	1.5561305	up	Tnut2
6872646 54447	0.008459655	4.26E-06	1.5533785	up	Asah2
6988194 66279	0.044974487	4.15E-04	1.5444175	down	Tmem218
6973472 243833	0.018898552	3.06E-05	1.5440156	up	Zfp128
6824507 67419	0.042421777	2.94E-04	1.5365113	up	3632451O06Rik
6883013 228858	0.042421777	3.33E-04	1.5346153	up	Gdap11
6779845 327900	0.022754725	5.74E-05	1.5159067	down	Ubd2
6762321 381290	0.042421777	3.39E-04	1.5038337	up	Atp2b4
6964250 68952	0.030455668	1.26E-04	1.5031539	down	Fam57b
6962751 381903	0.04098089	2.44E-04	1.4986535	down	Alg8
6805360 319181	0.02733521	7.69E-05	1.4921204	down	Hist1h2bg
6848581 106489	0.038548224	2.03E-04	1.4837484	down	Sf2d1 T2 Gm12166
6848581 100039624	0.038548224	2.03E-04	1.4837484	down	Sf2d1 T2 Gm12166
6872980 19662	0.022754725	4.55E-05	1.4830675	down	Rbp4
6983838 101966	0.022754725	4.20E-05	1.4753313	down	D8Ertid738e
6768261,6876138 432466	0.022754725	5.64E-05	1.473841	up	Gm5424 Ass1 Ass1 Gm5424
6768261,6876138 11898	0.022754725	5.64E-05	1.473841	up	Gm5424 Ass1 Ass1 Gm5424
6840887 207683	0.042421777	3.47E-04	1.4678116	down	Igsf11
6797969,6797978 13386	0.0259438	6.84E-05	1.4611462	down	Meg3 Dlk1 Mir1906 Dlk1
6852887 17685	0.04284105	3.77E-04	1.4595807	up	Msh2
6791995 71795	0.038548224	1.89E-04	1.4416575	down	Pitpnc1
6803780 67236	0.042421777	3.14E-04	1.4332547	down	Cinp
6752571 70829	0.022754725	5.59E-05	1.4327077	up	Code93
6877822 26877	0.043799955	3.88E-04	1.425908	down	B3galt1
6750351 108147	0.042421777	2.98E-04	1.4252509	up	Atic
6883267 110750	0.021531083	3.78E-05	1.4230214	up	Cse11
6886678 74194	0.030455668	1.22E-04	1.4227502	down	Rud3
6909629 67006	0.014083494	1.58E-05	1.4220327	down	Cisd2
6788815 11671	0.030455668	9.64E-05	1.4178725	up	Aldh3a2
6954385 13197	0.038548224	1.87E-04	1.4168825	down	Gadd45a Gng12
6769445 216198	0.042421777	3.55E-04	1.4158584	up	Tcp1l12
6798418 217944	0.022754725	4.49E-05	1.4127954	up	Rapgef5
6796305 56217	0.038548224	1.91E-04	1.4115229	up	Mpp5

6848581,6848584 21331	0.03972801	2.18E-04	1.4081773	down	Sft2d1 T2 Gm12166//T2
6902665 209601	0.042226546	2.71E-04	1.4075357	up	4922501L14Rik
6751538 67921	0.038548224	1.85E-04	1.4075081	down	Ube2f Gm5434
6916947 170638	0.030455668	1.10E-04	1.4067643	up	Hpcal4
6937047 67695	0.030455668	1.18E-04	1.4062134	down	Ost4 Agbl5
6972970 319748	0.017847234	2.76E-05	1.4012991	down	Zfp865 Zfp784 4632433K11Rik
6972970 654801	0.017847234	2.76E-05	1.4012991	down	Zfp865 Zfp784 4632433K11Rik
6972970 77043	0.017847234	2.76E-05	1.4012991	down	Zfp865 Zfp784 4632433K11Rik
6853388 70544	0.042226546	2.67E-04	1.400874	down	5730437N04Rik
6985850 68918	0.030455668	1.26E-04	1.3967563	down	1190005I06Rik
6896584 67576	0.042421777	2.85E-04	1.3956859	down	4930429B21Rik Zmat3
6899747,6899750,6899752,6907246,6907247 15077	0.049234077	5.02E-04	1.3945229	down	Hist2h2aa1 Hist2h2aa2 Hist2h2ac Hist2h3c1 Hist2h3c1- ps Hist2h3b Hist2h3c1 Hist2h3c2- ps Hist2h3c1 Hist2h3c2- ps Hist2h3b Hist2h2aa1 Hist2h2aa2 Hist2h3c1
6912947 108816	0.045647837	4.36E-04	1.3941127	down	4933409K07Rik Gm3893 Gm7819
6912947 100042539	0.045647837	4.36E-04	1.3941127	down	4933409K07Rik Gm3893 Gm7819
6912947 665845	0.045647837	4.36E-04	1.3941127	down	4933409K07Rik Gm3893 Gm7819
6890638 320961	0.042801354	3.66E-04	1.392631	down	Gabpb1 A630026N12Rik
6929651,6937047 231093	0.030455668	1.21E-04	1.388592	down	Agbl5 Ost4 Agbl5
6805380 319178	0.022754725	4.72E-05	1.3881029	down	Hist1h2bb
6782277 55984	0.039288376	2.12E-04	1.3869212	up	Camkk1
6918382,6918560 100503000	0.042421777	3.53E-04	1.3848228	up	Gm13051 Zfp534 1700029I01Rik Gm13251 Zfp600 Gm13242 Rex2 Gm13138 Gm13139 Gm13225 Gm13151 Gm13235 Gm13212 LOC100503000//1700029I01Rik Gm13251 Zfp534 Gm13139 Gm13151 2610305D13Rik LOC100503000
6839934 27406	0.030455668	1.20E-04	1.379296	up	Abcf3
6996440 235442	0.041485418	2.57E-04	1.3785135	up	Rab8b
6777309,6777310 17105	0.048785735	4.90E-04	1.3751312	up	Lyz2 Lyz1 Lyz1 Lyz2
6777309,6777310 17110	0.048785735	4.90E-04	1.3751312	up	Lyz2 Lyz1 Lyz1 Lyz2
6896584,6904047 22401	0.030455668	1.20E-04	1.3749123	down	4930429B21Rik Zmat3 Zmat3
6900404 99730	0.040309925	2.27E-04	1.3731047	down	Taf13
6917217 242667	0.042226546	2.73E-04	1.3728224	down	Dlgap3
6882768 228852	0.044817124	4.10E-04	1.3703306	down	Ppp1r16b
6751538,6794491 432649	0.04098089	2.45E-04	1.3591425	down	Ube2f Gm5434//Gm5434
6833308 56149	0.042421777	3.23E-04	1.3591031	down	Grasp
6797707 73046	0.044817124	4.08E-04	1.3571836	down	Girx5
6949826 30853	0.040628925	2.31E-04	1.3515993	down	Mlf2
6836699 23936	0.042801354	3.66E-04	1.3503007	down	Lynx1
6900239 81600	0.030455668	1.15E-04	1.3502584	up	Chia 1810022K09Rik
6900239 69126	0.030455668	1.15E-04	1.3502584	up	Chia 1810022K09Rik
6778425 11764	0.049234077	5.09E-04	1.3486375	up	Ap1b1
6758663 70396	0.049234077	5.02E-04	1.3484918	down	Asnsd1
6997077 71538	0.038548224	1.93E-04	1.343926	down	Fbxo9
6918382,6918397,6918560 1	0.042421777	3.14E-04	1.3402557	up	Gm13051 Zfp534 1700029I01

00043100					Rik Gm13251 Zfp600 Gm13242 Rex2 Gm13138 Gm13139 Gm13225 Gm13151 Gm13235 Gm13212 LOC100503000//Gm13157 1700029f01Rik Gm13251 Zfp534 Rex2 Gm13138 Gm13212 Gm13225 Gm13151 Gm13235 Gm13154//1700029f01Rik Gm13251 Zfp534 Gm13139 Gm13151 2610305D13Rik LOC100503000
6970138 55992	0.042421777	3.24E-04	1.3331729	up	Trim3
6979144,6985389 170737	0.026766581	7.34E-05	1.3291724	down	Znrf1//Ldhdl Znrf1
6966328 22282	0.042421777	2.89E-04	1.3234341	down	Ust2
6933409 71782	0.042421777	2.93E-04	1.3136151	up	Ankle2
6770923 327824	0.042421777	3.49E-04	1.3079915	down	5330438D12Rik LOC100504423
6770923 100504423	0.042421777	3.49E-04	1.3079915	down	5330438D12Rik LOC100504423
6978855 56513	0.046546645	4.52E-04	1.307966	down	Pard6a
6992219 56289	0.038548224	2.02E-04	1.3071496	down	Rassf1
6764048 641376	0.030455668	1.04E-04	1.3021629	down	Tom70l
6882521 66734	0.030455668	1.14E-04	1.3008461	down	Map11c3a
6873158 66583	0.044974487	4.17E-04	1.293808	down	Exosc1
6842933 74112	0.042421777	3.23E-04	1.2911134	down	Usp16
6921030,6921081,7003070,7003163 20301	0.04014609	2.23E-04	1.2910843	down	Ccl27a//Gm13306 Ccl27a//Zar1 Ccl27a
6921081,7003163 100039863	0.041485418	2.51E-04	1.2884337	down	Gm13306 Ccl27a
6980271 234023	0.042421777	3.41E-04	1.2866849	down	Argl1
6915929,6915993 13131	0.042421777	3.38E-04	1.2848579	down	Dab1 Gm10304 2900034C19Rik AY512949 LOC100502604//Dab1
6878995 56428	0.042226546	2.68E-04	1.2837576	down	Mtch2
6996704 21406	0.042421777	3.02E-04	1.2834076	up	Tcf12
6775250 28169	0.044817124	4.08E-04	1.2755362	down	Agpat3
6963972 59052	0.038548224	1.88E-04	1.2750655	down	Mettl9
6941761 207565	0.038548224	1.98E-04	1.268037	down	Camkk2
6771546,6777915 14421	0.042421777	3.22E-04	1.2680281	down	B4galnt1 Slc26a10//Slc26a10 B4galnt1
6771546,6777915 216441	0.042421777	3.22E-04	1.2680281	down	B4galnt1 Slc26a10//Slc26a10 B4galnt1
6964241 66162	0.042801354	3.73E-04	1.2623248	down	Bola2
6913863 72429	0.049234077	5.16E-04	1.2596972	down	Dnajc25 Gng10
6913863 14700	0.049234077	5.16E-04	1.2596972	down	Dnajc25 Gng10
6893279 18019	0.042421777	2.98E-04	1.2566174	down	Nfate2
6771538,6777902 12567	0.048785735	4.85E-04	1.2456908	down	Cdk4//Tspan31 Cdk4
6935486 56443	0.049234077	5.15E-04	1.2079331	up	Arpe1a

[00138] Table 5. Enriched pathways from Wikipathways altered with age in Drd1a-expressing striatal medium spiny neurons.

Pathway	p value	Matched Entities	Total Pathway Entities
Mm_XPodNet_-_protein-protein interactions in the podocyte expanded by STRING WP2309 72004	2.85E-05	13	836
Mm Chemokine signaling pathway WP2292 72463	9.62E-05	6	193
Mm PodNet- protein-protein interactions in the podocyte WP2310 72005	2.48E-04	7	315

Mm IL-7 Signaling Pathway WP297 69128	7.19E-04	3	44
Mm B Cell Receptor Signaling Pathway WP274 67072	0.003729099	4	156
Mm G Protein Signaling Pathways WP232 71315	0.005608093	3	91
Mm Integrin-mediated Cell Adhesion WP6 72138	0.006962605	3	101
Mm Striated Muscle Contraction WP216 72052	0.012084	2	45
Mm MAPK signaling pathway WP493 71754	0.024514528	3	159
Mm Purine metabolism WP2185 71316	0.02668426	3	178
Mm Primary Focal Segmental Glomerulosclerosis FSGS WP2573 72201	0.02678989	2	73
Mm Kit Receptor Signaling Pathway WP407 69079	0.030982522	2	67
Mm IL-5 Signaling Pathway WP151 69175	0.032727577	2	69

[00139] Table 6. Enriched pathways from Wikipathways altered with age in Drd2-expressing striatal medium spiny neurons.

Pathway	p value	Matched Entities	Total Pathway Entities
Mm_XPodNet_- protein-protein interactions in the podocyte expanded by STRING WP2309 72004	6.20E-10	51	836
Mm EGFR1 Signaling Pathway WP572 71756	3.89E-06	14	176
Mm PodNet- protein-protein interactions in the podocyte WP2310 72005	1.12E-05	18	315
Mm MAPK signaling pathway WP493 71754	1.07E-04	11	159
Mm Myometrial Relaxation and Contraction Pathways WP385 72108	1.43E-04	11	157
Mm Hypothetical Network for Drug Addiction WP1246 69102	1.72E-04	5	32
Mm Calcium Regulation in the Cardiac Cell WP553 73390	3.56E-04	10	150
Mm IL-6 signaling Pathway WP387 72091	4.08E-04	8	99
glutathione redox reactions I	4.82E-04	3	9
Mm B Cell Receptor Signaling Pathway WP274 67072	5.18E-04	10	156
Mm Primary Focal Segmental Glomerulosclerosis FSGS WP2573 72201	9.00E-04	6	73
glutathione-mediated detoxification	9.06E-04	4	24
Mm IL-7 Signaling Pathway WP297 69128	0.001013867	5	44
Mm ErbB signaling pathway WP1261 71282	0.001013867	5	46
Mm G Protein Signaling Pathways WP232 71315	0.001068723	7	91
Mm Estrogen signalling WP1244 73501	0.001363316	6	74
Mm Kit Receptor Signaling Pathway WP407 69079	0.001363316	6	67
Mm Amino Acid metabolism WP662 71177	0.001488036	7	95
Mm MAPK Cascade WP251 71729	0.001646583	4	29
Mm Integrin-mediated Cell Adhesion WP6 72138	0.001687754	7	101
Mm Insulin Signaling WP65 71726	0.001848077	9	159
Mm Splicing factor NOVA regulated synaptic proteins WP1983 71717	0.002140725	4	42
Mm Cholesterol Biosynthesis WP103 71741	0.002402918	3	15
gluconeogenesis I	0.002917192	3	17
GDP-mannose biosynthesis I	0.003268231	2	6
GDP-mannose biosynthesis	0.003268231	2	6
Mm Oxidative Damage WP1496 75225	0.00380393	4	41
Mm Urea cycle and metabolism of amino groups WP426 72149	0.004844879	3	37
Mm G1 to S cell cycle control WP413 72012	0.004965064	5	62
Mm Selenium Micronutrient Network WP1272 73551	0.005622589	3	31
Mm TGF-beta Receptor Signaling Pathway WP258 73847	0.006082267	8	150
Mm Eukaryotic Transcription Initiation WP567 69915	0.006176465	4	41
Mm Folic Acid Network WP1273 74467	0.006470848	3	27
Mm Tryptophan metabolism WP79 73389	0.007349561	4	44
fatty acid Beta-oxidation I	0.007391186	3	24
Mm Wnt Signaling Pathway and Pluripotency WP723 69165	0.007550718	6	97
spermine biosynthesis II	0.008820865	2	8
superpathway of D-myo-inositol (1,4,5)-trisphosphate metabolism	0.008820865	2	8
Mm Exercise-induced Circadian Regulation WP544 69890	0.011720306	4	49
Mm Metapathway biotransformation WP1251 69747	0.011818458	3	143

Mm IL-2 Signaling Pathway WP450 67368	0.011883704	5	76
pyrimidine ribonucleotides interconversion	0.013834631	2	10
Mm miRNAs involved in DNA damage response WP2085 74241	0.013834631	2	49
pyrimidine ribonucleotides de novo biosynthesis	0.016704416	2	12
CDP-diacylglycerol biosynthesis I	0.016704416	2	13
Mm Regulation of Actin Cytoskeleton WP523 71326	0.017078303	7	151
Mm Prostaglandin Synthesis and Regulation WP374 69204	0.019096008	3	31
Mm Cell cycle WP190 71755	0.01963693	5	88
phosphatidylglycerol biosynthesis II (non-plastidic)	0.019803159	2	14
Mm Glycogen Metabolism WP317 70007	0.020789187	3	34
Mm Signaling of Hepatocyte Growth Factor Receptor WP193 69178	0.022562083	3	34
starch degradation	0.023121472	2	14
colanic acid building blocks biosynthesis	0.023121472	2	14
fatty acid Beta-oxidation II (core pathway)	0.023121472	2	15
Mm SIDS Susceptibility Pathways WP1266 69139	0.024744025	4	61
tRNA charging pathway	0.026346961	3	37
glycolysis III	0.026650239	2	14
Mm T Cell Receptor Signaling Pathway WP480 69149	0.027621077	6	133
glycolysis I	0.030380595	2	16
Mm PluriNetWork WP1763 72003	0.035232157	10	291
Mm Striated Muscle Contraction WP216 72052	0.037194125	3	45
Mm IL-3 Signaling Pathway WP373 69196	0.037842713	5	100
Mm Nucleotide Metabolism WP87 71749	0.047149517	2	19
Mm Glutathione metabolism WP164 71334	0.047149517	2	19
Mm Wnt Signaling Pathway NetPath WP539 71716	0.04984857	5	109
Mm Selenium metabolism-Selenoproteins WP108 69772	0.049974676	3	48

[00140] Table 7. Enriched pathways from Wikipathways altered with age in Drd1a-expressing cortical neurons.

Pathway	p value	Matched Entities	Total Pathway Entities
Mm Striated Muscle Contraction WP216 72052	1.49E-04	3	45
Mm Keap1-Nrf2 WP1245 71125	4.95E-04	2	14
Mm Fatty Acid Biosynthesis WP336 71737	0.001443061	2	22
Mm Signaling of Hepatocyte Growth Factor Receptor WP193 69178	0.003238718	2	34
bupropion degradation	0.003435435	2	35
nicotine degradation III	0.005203122	2	43
nicotine degradation II	0.006749277	2	49
Mm B Cell Receptor Signaling Pathway WP274 67072	0.006916409	3	156
Mm Myometrial Relaxation and Contraction Pathways WP385 72108	0.007299635	3	157
Mm Primary Focal Segmental Glomerulosclerosis FSGS WP2573 72201	0.010716978	2	73
Mm IL-2 Signaling Pathway WP450 67368	0.015482554	2	76
Mm_XPodNet_-_protein-protein interactions in the podocyte expanded by STRING WP2309 72004	0.015918477	6	836
Mm IL-6 signaling Pathway WP387 72091	0.025211193	2	99

[00141] Table 8. Enriched pathways from Wikipathways altered with age in Drd2-expressing cortical neurons.

Pathway	p value	Matched Entities	Total Pathway Entities
Mm_XPodNet_-_protein-protein interactions in the podocyte expanded by STRING WP2309 72004	9.61E-05	12	836
Mm B Cell Receptor Signaling Pathway WP274 67072	0.003393002	4	156

glutathione-mediated detoxification	0.004241159	2	24
Mm Prostaglandin Synthesis and Regulation WP374_69204	0.007014286	2	31
Mm Retinol metabolism WP1259_74433	0.010410202	2	39
Mm Striated Muscle Contraction WP216_72052	0.011489692	2	45
Mm Adipogenesis genes WP447_73875	0.01569575	3	133
Mm G1 to S cell cycle control WP413_72012	0.024738263	2	62
Mm Chemokine signaling pathway WP2292_72463	0.034508925	3	193
Mm Cell cycle WP190_71755	0.04576582	2	88

[00142] Table 9. . Lentiviruses used in this study

95 shRNA lentiviruses targeting 76 distinct target sequences

Hairpin ID	Vector Name	Transcript Targeted	Gene Symbol	Target type	Reason Gene was Chosen	NCBI Gene ID	Target Region	Target Sequence
TRCN0000072261	pLKO.1	promegaLuc.1	Luciferase	Control	Negative Control		CDS	CACTCGGATATTGGATATGTG
TRCN0000072250	pLKO.1	promegaLuc.1	Luciferase	Control	Negative Control		CDS	AGAATCGTCGTATGCAGTGAA
TRCN0000066072	pLKO.1	NM_134101.1	Psmc2	Control	Positive Control	21762	CDS	CGCCAGTTAGCTCAATATCAT
TRCN0000207065	pLKO.1	clonetechnGfp.1	GFP	Control	Negative Control		CDS	GCGATCACATGGTCCTGCTGG
TRCN0000072231	pLKO.1	lacZ.1	LacZ	Control	Negative Control		CDS	CGCTAAATACTGGCAGGCGTT
TRCN0000072209	pLKO.1	rfp.1	RFP	Control	Negative Control		CDS	CTCAGTTCAGTACGGCTCCA
TRCN0000072181	pLKO.1	clonetechnGfp.1	GFP	Control	Negative Control		CDS	ACAACAGCCACAACGTCTATA
TRCN0000231782	pLKO_TRC021	None	None	Control	Negative Control	None	Non-shRNA transcript	ACAGTTAACCACCTTTTGAAT
TRCN0000428544	pLKO_TRC005	NM_053139.3	Pcdhb14	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93885	CDS	GTAGTGCAACCATCACGTATT
TRCN0000435247	pLKO_TRC005	NM_053139.3	Pcdhb14	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93885	CDS	AGGCAAGTGACCGCCATTATC
TRCN0000419614	pLKO_TRC005	NM_053139.3	Pcdhb14	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93885	3'UTR	CATGATACTGGTAGTCATATT
TRCN0000426134	pLKO_TRC005	NM_053139.3	Pcdhb14	Experimental	Upregulated with age in	93885	CDS	TCAGTACTTATCAGCGAAATT

					Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)			
TRCN0000320173	pLKO_TRC005	NM_010517.3	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002) and Igfbp4 is striatal-enriched (Heiman et al., 2008)	16010	CDS	CATTCCAAACTGTGACCGCAA
TRCN0000350214	pLKO_TRC005	NM_010517.3	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002) and Igfbp4 is striatal-enriched (Heiman et al., 2008)	16010	CDS	GCTGCGGTTGTTGCGCCACTT
TRCN0000114798	pLKO.1	NM_010517.2	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002) and Igfbp4 is striatal-enriched (Heiman et al., 2008)	16010	CDS	GACAAGGATGAGAGCGAACAT
TRCN0000114797	pLKO.1	NM_010517.2	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002) and Igfbp4 is striatal-enriched (Heiman et al., 2008)	16010	CDS	CATTCCAAACTGTGACCGCAA
TRCN0000114800	pLKO.1	NM_010517.2	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002) and Igfbp4 is striatal-enriched (Heiman et al., 2008)	16010	CDS	GCTGCGGTTGTTGCGCCACTT
TRCN0000320111	pLKO_TRC005	NM_010517.3	Igfbp4	Experimental	IGF-1 has neuroprotective effects in HD (Humbert et al., 2002)	16010	CDS	GACAAGGATGAGAGCGAACAT

					and Igfbp4 is striatal-enriched (Heiman et al., 2008)			
TRCN0000288175	pLKO_TRC005	NM_011063.2	Pea15a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	18611	CDS	CAAAGACAACCTCTCCTACAT
TRCN0000105789	pLKO.1	NM_011063.1	Pea15a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	18611	CDS	CCTGACCAACAACATCACCT
TRCN0000105787	pLKO.1	NM_011063.1	Pea15a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	18611	CDS	CAAAGACAACCTCTCCTACAT
TRCN0000288240	pLKO_TRC005	NM_011063.2	Pea15a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	18611	CDS	CCTGACCAACAACATCACCT
TRCN0000307569	pLKO_TRC005	NM_011063.2	Pea15a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	18611	CDS	ACACCAAGCTAACCCTATTC
TRCN0000096379	pLKO.1	NM_007488.2	Arnt2	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	11864	3UTR	CGCTATTATCATGCCATAGAT
TRCN0000096382	pLKO.1	NM_007488.2	Arnt2	Experimental	Upregulated with age in Drd1a- and Drd2-	11864	CDS	CCTACTCTGATGAGATCGAGT



					expressing medium spiny neurons (Tables S1 and S2)			
TRCN0000323726	pLKO_TRC005	NM_007488.2	Arnt2	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	11864	3UTR	CGCTATTATCATGCCATAGAT
TRCN0000323788	pLKO_TRC005	NM_007488.2	Arnt2	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	11864	CDS	CCTACTCTGATGAGATCGAGT
TRCN0000374677	pLKO_TRC005	NM_007488.2	Arnt2	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	11864	CDS	TGTCGGACAAGGCAGTAAATA
TRCN0000231232	pLKO_TRC005	NM_001038695.1	Kdm3a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	104263	CDS	CACGATCAGAGCTGGTATTTA
TRCN0000252744	pLKO_TRC005	NM_001038695.2	Kdm3a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	104263	CDS	TGCGGGTAGAAGGCTTCTTAA
TRCN0000252745	pLKO_TRC005	NM_001038695.2	Kdm3a	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	104263	3UTR	CTGCGAAGTTTCGTTGGATTT
TRCN0000252747	pLKO_TRC005	NM_001038695.2	Kdm3a	Experimental	Upregulated with age in Drd1a- and	104263	CDS	GAAGTTCTGAGCAAGTTATT

					Drd2-expressing medium spiny neurons (Tables S1 and S2)			
TRCN0000295705	pLKO_TRC005	NM_009735.3	B2m	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	12010	3UTR	CCAGTTTCTAATAFGCTATAC
TRCN0000295762	pLKO_TRC005	NM_009735.3	B2m	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	12010	CDS	TAAAGTAGAGATGTCAGATAAT
TRCN0000288438	pLKO_TRC005	NM_009735.3	B2m	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	12010	CDS	GCCGAACATACTGAACTGCTA
TRCN0000066424	pLKO.1	NM_009735.2	B2m	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	12010	CDS	GCCGAACATACTGAACTGCTA
TRCN0000329356	pLKO_TRC005	NM_177386.4	Sfmbt2	Experimental	Previously shown to change in published HD studies (Becanovic	353282	CDS	CCCTCTGACCACACCATATAA

TRCN0000329354	pLKO_TRC005	NM_177386.4	Sfmbt2	Experimental	et al., 2010) Previously shown to change in published HD studies (Becanovic et al., 2010)	353282	CDS	CGGATGTGGTACGATTCATTA
TRCN0000329357	pLKO_TRC005	NM_177386.4	Sfmbt2	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	353282	3UTR	CCTATTTGATAGTCCTATATT
TRCN0000329285	pLKO_TRC005	NM_177386.4	Sfmbt2	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	353282	CDS	TTCGTCAACCACCGGTGTTTC
TRCN0000337555	pLKO_TRC005	NM_030143.4	Ddit4l	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	73284	3UTR	CCCTAATGAGTGGATAATAAAA
TRCN0000276917	pLKO_TRC005	NM_030143.3	Ddit4l	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	73284	CDS	GATTCGACTACTGGGATTAT
TRCN0000176976	pLKO.1	NM_030143.2	Ddit4l	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	73284	CDS	GATTCGACTACTGGGATTAT
TRCN0000276918	pLKO_TRC005	NM_030143.3	Ddit4l	Experimental	Previously shown to change in published HD studies (Becanovic et al., 2010)	73284	CDS	TCGCTTCTCCTCAGGCCCTTAA
TRCN0000183203	pLKO.1	NM_010726.1	Phyh	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	16922	3UTR	GAGGACATCAAAGCAAAGAA A
TRCN0000183360	pLKO.1	NM_010726.1	Phyh	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1	16922	3UTR	GCTCTCCTTATAAATTCCTTT

TRCN0000314263	pLKO_TRC005	NM_010726.2	Phyh	Experimental	Upregulated with age in Drd1a- and Drd2- expressing medium spiny neurons (Tables S1 and S2)	16922	3UTR	GAGGACATCAAAGCAAAGAA A
TRCN0000314262	pLKO_TRC005	NM_010726.2	Phyh	Experimental	Upregulated with age in Drd1a- and Drd2- expressing medium spiny neurons (Tables S1 and S2)	16922	3UTR	GCCTCTTCCTTATAAATTCCTTT
TRCN0000221761	pLKO.1	NM_008828.1	Pgk1	Experimental	Randomly chosen housekeeping target gene	18655	CDS	CATCAAATTCCTGCTTGGACAA
TRCN0000104502	pLKO.1	NM_009094.1	Rps4x	Experimental	Proteins involved in translation have been shown to be associated with Huntingtin protein (Culver et al., 2012)	20102	CDS	CCCTGACTGGAGATGAAGTAA
TRCN0000104426	pLKO.1	NM_016980.1	Rpl5	Experimental	Proteins involved in translation have been shown to be associated with Huntingtin protein (Culver et al., 2012)	100503 670	CDS	CCCTCATAGTACCAAACGATT
TRCN0000311277	pLKO_TRC005	NM_009483.1	Kdm6a	Experimental	Upregulated with age when Drd1a- and Drd2- expressing medium spiny neuron gene expression data are pooled (analysis not shown)	22289	3UTR	CTATGCCAGGACTCTCGTAAA
TRCN0000305239	pLKO_TRC005	NM_009483.1	Kdm6a	Experimental	Upregulated with age when Drd1a- and Drd2- expressing medium spiny neuron gene expression	22289	CDS	AGTTAGCAGTGGAAACGTTATG

					data are pooled (analysis not shown)			
TRCN0000096242	pLKO.1	NM_009483.1	Kdm6a	Experimental	Upregulated with age when Drd1a- and Drd2- expressing medium spiny neuron gene expression data are pooled (analysis not shown)	22289	CDS	GCTACGAATCTCTAATCTTAA
TRCN0000331919	pLKO_TRC005	NM_009483.1	Kdm6a	Experimental	Upregulated with age when Drd1a- and Drd2- expressing medium spiny neuron gene expression data are pooled (analysis not shown)	22289	CDS	GCTACGAATCTCTAATCTTAA
TRCN0000085087	pLKO.1	NM_025444.1	Taf13	Experimental	Downregulated with age in Drd1a- and Drd2- expressing medium spiny neurons, as well as Drd2- expressing cortical cells (Tables S1, S2, and S4)	99730	CDS	CGAAGACCTTGTCATAGAGTT
TRCN0000085085	pLKO.1	NM_025444.1	Taf13	Experimental	Downregulated with age in Drd1a- and Drd2- expressing medium spiny neurons, as well as Drd2- expressing cortical cells (Tables S1, S2, and S4)	99730	CDS	AGAATTGAAACGGGCTAGAAA
TRCN0000317962	pLKO_TRC005	NM_025444.2	Taf13	Experimental	Downregulated with age in Drd1a- and Drd2- expressing medium spiny neurons, as well as Drd2- expressing	99730	CDS	CGAAGACCTTGTCATAGAGTT

					cortical cells (Tables S1, S2, and S4)			
TRCN0000317963	pLKO_TRC005	NM_025444.2	Taf13	Experimental	Downregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	99730	CDS	AGAATTGAAACGGGCTAGAAA
TRCN0000287596	pLKO_TRC005	NM_026163.2	Pkp2	Experimental	Randomly chosen target gene	67451	CDS	GCCTTGAGAAACTTGGTATTT
TRCN0000123350	pLKO.1	NM_026163.1	Pkp2	Experimental	Randomly chosen target gene	67451	CDS	GCCTTGAGAAACTTGGTATTT
TRCN0000123351	pLKO.1	NM_026163.1	Pkp2	Experimental	Randomly chosen target gene	67451	CDS	CCTGAGTATGTCTACAAGCTA
TRCN0000287514	pLKO_TRC005	NM_026163.2	Pkp2	Experimental	Randomly chosen target gene	67451	CDS	CCTGAGTATGTCTACAAGCTA
TRCN0000071993	pLKO.1	NM_053242.3	Foxp2	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S2 and S4)	114142	3UTR	CGGAAGTTATTGATGTGGTAT
TRCN0000071994	pLKO.1	NM_053242.3	Foxp2	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S2 and S4)	114142	CDS	CGGACAGTCTTCAGTTCTGAA
TRCN0000071997	pLKO.1	NM_053242.3	Foxp2	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S2 and S4)	114142	CDS	GCGACATTCAGACAAATACAA
TRCN0000076492	pLKO.1	NM_145451.1	Gpx6	Experimental	Upregulated	75512	CDS	AGCCATTCAACGTCACGGTTT

					with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)			
TRCN0000076491	pLKO.1	NM_145451.1	Gpx6	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	75512	CDS	GTGAACGGAGACAATGAACAA
TRCN0000076488	pLKO.1	NM_145451.1	Gpx6	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	75512	3'UTR	GCATGTGCAATCTACAGAGAT
TRCN0000125009	pLKO.1	NM_177346.1	Gpr149	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	229357	3'UTR	CCCACITTCITCTAGTTATAT
TRCN0000125011	pLKO.1	NM_177346.1	Gpr149	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	229357	CDS	GCGATATTAACATATGGAGAAA
TRCN0000125010	pLKO.1	NM_177346.1	Gpr149	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	229357	CDS	CCAGTGITTTGCTTATCCAAA
TRCN0000317130	pLKO_TRC005	NM_009112.2	Sl00a10	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	20194	CDS	CCAGAGCTTCTATCACTAGT
TRCN0000097669	pLKO.1	NM_009112.1	Sl00a10	Experimental	Upregulated with age in	20194	CDS	CCAGAGCTTCTATCACTAGT

					Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)			
TRCN0000034382	pLKO.1	NM_008284.1	Hras1	Experimental	Downregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	15461	CDS	CGGGTGAAAGATTCAGATGAT
TRCN0000036695	pLKO_TRC005	NM_008284.2	Hras1	Experimental	Downregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	15461	CDS	GTGAGATTCGGCAGCATAAAAT
TRCN0000036696	pLKO_TRC005	NM_008284.2	Hras1	Experimental	Downregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	15461	3UTR	CACGTTGCATCACAGTAAATF
TRCN00000323443	pLKO_TRC005	NM_019635.2	Sik3	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	56274	CDS	CCTGAGGTAATCAAGAAATA
TRCN0000025880	pLKO.1	NM_019635.1	Stk3	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1,	56274	CDS	CCTTCTTTCATGGACTACTTT



TRCN0000025951	pLKO.1	NM_019635.1	Skk3	Experimental	S2, and S4) Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons, as well as Drd2-expressing cortical cells (Tables S1, S2, and S4)	56274	CDS	CCTGAGGTAATTCAAGAAATA
TRCN0000094178	pLKO.1	NM_053133.1	Pcdhb8	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	93879	CDS	AGACTTGCAGTTCACAGATAT
TRCN0000094175	pLKO.1	NM_053133.1	Pcdhb8	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	93879	CDS	CTGGCTCCAATGGCCTTATTA
TRCN0000094176	pLKO.1	NM_053133.1	Pcdhb8	Experimental	Upregulated with age in Drd2-expressing medium spiny neurons (Table S2)	93879	CDS	CACAGATATAAACGACCATT
TRCN0000077330	pLKO.1	NM_028810.1	Rnd3	Experimental	Downregulated with age in all cell types studied (Tables S1, S2, S3, and S4)	74194	CDS	GCACATTAGTGGAACCTCTCAA
TRCN0000331730	pLKO_TRC005	NM_028810.2	Rnd3	Experimental	Downregulated with age in all cell types studied (Tables S1, S2, S3, and S4)	74194	CDS	GCACATTAGTGGAACCTCTCAA
TRCN0000081679	pLKO.1	NM_010118.1	Egr2	Experimental	Downregulated with age in Drd1a-expressing medium spiny neurons (Table S1)	13654	CDS	CCACTCTCTACCATCCGTAAT
TRCN0000235775	pLKO_TRC005	NM_010118.3	Egr2	Experimental	Downregulated with age in Drd1a-expressing medium spiny neurons (Table S1)	13654	CDS	GAGATGGCATGATCAACATTG

TRCN0000427699	pLKO_TRC005	NM_053131.1	Pcdh6	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93877	CDS	GCTCACACTCTACCTGGTCAT
TRCN0000434269	pLKO_TRC005	NM_053131.1	Pcdh6	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93877	CDS	CAAATTCCTGAACCAATTATTC
TRCN0000094302	pLKO.1	NM_053131.1	Pcdh6	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	93877	CDS	CCAGAAATGCTTGGCTGTCATT
TRCN0000430303	pLKO_TRC005	NM_172126.2	Adam1a	Experimental	Randomly chosen target gene	280668	CDS	TTGCGCAACATGTACGCTTAA
TRCN0000031725	pLKO.1	NM_172126.2	Adam1a	Experimental	Randomly chosen target gene	280668	CDS	GCACACAGTGTGATAGGATTT
TRCN0000438367	pLKO_TRC005	NM_199021.3	Dpp10	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	269109	CDS	GGCATCCAGTGTACTGCATAA
TRCN0000031459	pLKO.1	NM_199021.2	Dpp10	Experimental	Upregulated with age in Drd1a- and Drd2-expressing medium spiny neurons (Tables S1 and S2)	269109	3'UTR	GCTTCFTTATTGAGCCAAATA
TRCN0000104268	pLKO.1	NM_052835.1	Rpl10	Experimental	Proteins involved in translation have been shown to be associated with Huntingtin protein (Culver et al., 2012)	110954	CDS	CCGAACCAAGTTGCAGAACAA

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[00143] Table 10. Log2 sequencing results from the SLIC screen time-points

4 replicates per time-point and genotype  
 95 viral elements targeting 76 distinct target sequences

Hairpin Sequence	Hairpin IDs	Gene Name
CGCCAGTTAGCTCAATATCAT	TRCN0000066072	Psmc2 (positive control)
AGACTTGCAGTTCACAGATAT	TRCN0000094178	Pcdhb8
CCTGAGGTAATFCAAGAAATA	TRCN0000025951,TRCN0000323443	Stk3
GCAATGTGCAATCTACAGAGAT	TRCN0000076488	Gpx6
TAAAGTAGAGATGTCAGATAT	TRCN0000295762	B2m
GTGAACGGAGACAATGAACAA	TRCN0000076491	Gpx6
CTGCGAAGTTTCGTTGGATTT	TRCN0000252745	Kdm3a
CCTGACCAACAACATCACCCCT	TRCN0000105789,TRCN0000288240	Pea15a
CAAAGACAACCTCTCTACAT	TRCN0000105787,TRCN0000288175	Pea15a
CCCTGACTGGAGATGAAGTAA	TRCN0000104502,TRCN0000316606	Rps4x
CCCTCATAGTACCAAACGATT	TRCN0000104426	Rpl5
AGCCATTC AACGTCACGGTTT	TRCN0000076492	Gpx6
CACAGATATAAACGACCATT	TRCN0000094176	Pcdhb8
CAAATTCCTGAAACCATTATC	TRCN0000434269	Pcdhb6
ACACCAAGCTAACCCGTATTC	TRCN0000307569	Pea15a
CTATGCCAGGACTCTCGTAAA	TRCN0000311277	Kdm6a
TGTCGGACAAGGCAGTAAATA	TRCN0000374677	Arnt2
CACGTTGCATCACAGTAAATT	TRCN0000366696	Hras1
CGCTAAATACTGGCAGGC GTT	TRCN0000072231,TRCN0000231710	LacZ (negative control)
GCCTTGAGAAACTTGGTATTT	TRCN0000123350,TRCN0000287596	Pkp2
CTATTGTGATAGTCTATAT	TRCN0000329357	Sfmbt2
CCTTCTTTCATGGACTACTTT	TRCN0000025880	Stk3
TTCGCCAACATGTACGCTTAA	TRCN0000430303	Adam1a
CCGAACCAAGTTGCAGAACAA	TRCN0000104268	Rpl10
CACTCGGATATTTGATATGTG	TRCN0000072261,TRCN0000231707	Luciferase (negative control)
CCTGAGTATGCTACAAGCTA	TRCN0000123351,TRCN0000287514	Pkp2
CCAGTTTCTAATATGCTATAC	TRCN0000295705	B2m
CCCTAATGAGTGGATAATAAA	TRCN0000337555	Ddit4l
GGCATCCAGTGTACTGCATAA	TRCN0000438367	Dpp10
AGGCAAGTGACCGCCATFATC	TRCN0000435247	Pcdhb14
GCTCTTCTTATAATTCCTTT	TRCN0000183360,TRCN0000314262	Phyh
TGCGGGTAGAAGGCTTCTTAA	TRCN0000252744	Kdm3a
TCGCTTCTCTCAGGCCTTAA	TRCN0000276918	Ddit4l

CCACTCTCTACCATCCGTAAT	TRCN0000081679	Egr2
CATCAAATTCGCTTGGACAA	TRCN0000221761	Pgk1
CCCCTTTCTTCTAGTTATAT	TRCN0000125009	Gpri49
CTGGCTCCAATGGCCTTATTA	TRCN0000094175	Pcdhb8
GCGATATTAACATATGGAGAAA	TRCN0000125011	Gpri49
CATTCCAAACTGTGACCGCAA	TRCN0000114797,TRCN0000320173	Igfbp4
CCFACTCTGATGAGATCGAGT	TRCN0000096382,TRCN0000323788	Arnt2
CCAGAGCTTTCTATCACTAGT	TRCN0000097669,TRCN0000317130	Sl00a10
CGAAGACCTTGTATAGAGTT	TRCN0000085087,TRCN0000317962	Taf13
AGAATFGAAACGGGCTAGAAA	TRCN0000085085,TRCN0000317963	Taf13
CCCTCTGACCACACCATATAA	TRCN00000329356	Sfmbt2
CCAGAATGCTTGGCTGTCAAT	TRCN0000094302	Pcdhb6
GATTTGACTACTGGGATTAT	TRCN0000176976,TRCN0000276917	Ddn4l
GAGGACATCAAAGCAAAGAAA	TRCN0000183203,TRCN0000314263	Phyh
GCCACAGTGTGATAGGATTT	TRCN0000031725	Adam1a
CGCTATTATCATGCCATAGAT	TRCN0000096379,TRCN0000323726	Arnt2
CAGGATCAGAGCTGGTATTTA	TRCN0000231232	Kdm3a
AGAATCGTCGTATGCAAGTAAA	TRCN0000072250,TRCN0000231730	Luciferase (negative control)
GCGACATTCAGACAAAATACAA	TRCN0000071997	Foxp2
GCCATTAGTGGAACTCTCAA	TRCN0000077330,TRCN0000331730	Rnd3
GCTACGAATCTCTAATCTTAA	TRCN0000096242,TRCN0000331919	Kdm6a
CCAGTGTTFGTCCTTATCCAAA	TRCN0000125010	Gpri49
ACAACAGCCACAACGTCCTATA	TRCN0000464743,TRCN0000464744,TRCN0000464745,TRCN0000464747,TRCN0000072181,TRCN0000231753	GFP (negative control)
CGGACAGTCTTCAGTCTTGAA	TRCN0000071994	Foxp2
GAAGTTCCTGAGCAAGTTATT	TRCN0000252747	Kdm3a
GTAGTGCAACCATCACGTATT	TRCN0000428544	Pcdhb14
TCAGTACTTATCAGCGAAATT	TRCN0000426134	Pcdhb14
TTCGTCAACCACGGTGTTC	TRCN0000329285	Sfmbt2
CGGAAGTTATTGATGTGGTAT	TRCN0000071993	Foxp2
GCTCACACTTACCTGGTCAT	TRCN0000427699	Pcdhb6
CGGATGTGGTACGATTCATTA	TRCN0000329354	Sfmbt2
AGTFAGCAGTGGAAACGTTATG	TRCN0000305239	Kdm6a
GAGATGGCATGATCAACATFG	TRCN0000235775	Egr2
GACAAGGATGAGAGCGAACAT	TRCN0000114798,TRCN0000320111	Igfbp4
GCGATCACATGGTCTGTCTGG	TRCN0000207065	GFP (negative control)
CTCAGTTCACGATACGGCTCCA	TRCN0000072209,TRCN0000231683	RFP (negative control)
GCTTCTTTATTGAGCCAAATA	TRCN0000031459	Dpp10
GCTGCGGTTGTTGCGCCACTT	TRCN0000114800,TRCN0000350214	Igfbp4
GCCGAACATACTGAACTGCTA	TRCN0000066424,TRCN0000288438	B2m
GTGAGATTGGCAGCATAAAT	TRCN0000366695	Hras1
CGGGTGAAGATTTCAGATGAT	TRCN0000034382	Hras1
CATGATACTGGTAGTCATATT	TRCN0000419614	Pcdhb14
ACAGTTAACCACITTTTGAAT	TRCN0000464725,TRCN0000464728,TRCN0000464730,TRCN0000464732,TRCN0000464733,TRCN0000464734,TRCN0000464735,TRCN0000464736,TRCN0000464738,TRCN0000241922,TRCN0000464737,TRCN0000464741,TRCN0000464742,TRCN0000241923,TRCN0000231782,TRCN0000464726,TRCN0000464727,TRCN0000464729,TRCN0000464731,TRCN0000464723,TRCN0000464724	shRNA negative control (non-shRNA transcript, negative control)

[00144] Table 11. RIGER-assigned p values for depletion in the SLIC screen at 4 weeks.

Gene	Hairpins	# Hairpins	Hairpin ranks	Normalized enrichment score	Gene rank	p value	p value rank
Gpx6	GCATGTGCAATCTACAGAGAT, GTGAACGGAGACAATGAACAA, AGCCATTCAACGTCACGGTTT	3	9, 3, 2	0.05882	1	0.0036	1
Pcdhb8	CTGGCTCCAATGGCCTTATTA, CACAGATATAAACGACCATTT, AGACTTGCAGTTCACAGATAT	3	13, 16, 4	0.2299	2	0.083	2

Pkp2	GCCTTGAGAAACTTGGTATTT, CCTGAGTATGTCTACAAGCTA	2	24, 30	0.5089	5	0.2377	3
Gpr149	CCCACCTTCTCTAGTTATAT, CCAGTGTGTTGCTTATCCAAA, GCGATATTAACATATGGAGAAA	3	22, 37, 12	0.4171	3	0.2566	4
Taf13	AGAATTGAAACGGGCTAGAAA, CGAAGACCTTGTTCATAGAGTT	2	17, 34	0.5312	6	0.2606	5
Phyh	GCTCTTCCTTATAAATTCCTTT, GAGGACATCAAAGCAAAGAAA	2	19, 35	0.5536	7	0.2827	6
Hras1	CGGGTGAAAGATTTCAGATGAT, CACGTTGCATCACAGTAAATT, GTGAGATTCCGGCAGCATAAAT	3	74, 18, 23	0.4652	4	0.3083	7
GFP	ACAACAGCCACAACGTCTATA, GCGATCACATGGTCCTGCTGG	2	36, 25	0.5938	8	0.3282	8
LUCIFERASE	AGAATCGTTCGTATGCAGTGAA, CACTCGGATATTTGATATGTG	2	20, 40	0.625	9	0.3619	9
Foxp2	CGGAAGTTATTTGATGTGGTAT, CGGACAGTCTTCAGTTCTGAA, GCGACATTCAGACAAATACAA	3	41, 21, 33	0.6417	10	0.5203	10
Stk3	CCTTCTTTCATGGACTACTTT, CCTGAGGTAATTTCAAGAAATA	2	63, 7	0.875	15	0.7267	11
Igfbp4	CATTCCAAACTGTGACCGCAA, GACAAGGATGAGAGCGAACAT, GCTGCGGTTGTTGCGCCACTT	3	54, 48, 8	0.8128	11	0.727	12
Ddit4l	GATTTGCGACTACTGGGATPAT, TCGCTTCTCCTCAGGCCTTAA, CCCTAATGAGTGGATAATAAAA	3	46, 15, 53	0.8182	12	0.7335	13
Peal5a	CAAAGACAACCTCTCCTACAT, CCTGACCAACAACATCACCTT, ACACCAAGCTAACCCGTATTC	3	39, 38, 44	0.8289	13	0.7465	14
Arnt2	CGCTATTATCATGCCATAGAT, CCTACTCTGATGAGATCGAGT, TGTCGGACAAGGCAGTAAATA	3	31, 42, 68	0.8396	14	0.759	15
Podhb6	CCAGAATGCTTGGCTGTCATT, CAAATTCCTGAACCATTATTC, GCTCACACTCTACCTGGTCAT	3	14, 61, 52	0.9091	16	0.8288	16
Dpp10	GCTTCTTTATTGAGCCAAATA, GGCATCCAGTGTACTGCATAA	2	56, 43	0.942	17	0.832	17
B2m	GCCGAACATACTGAACTGCTA, TAAAGTAGAGATGTCAGATAT, CCAGTTTCTAATATGCTATAC	3	64, 11, 57	0.9733	18	0.8837	18
Podhb14	TCAGTACTTATCAGCGAAATT, CATGATACTGGTAGTCATATF, GTAGTGCAACCATCACGTATT, AGGCAAGTGACCGCCATTATC	4	75, 60, 72, 10	1.2025	19	0.9765	19
Kdm3a	CACGATCAGAGCTGGTATTTA, CTGCGAAGTTTCGTTGGATTT, TGCGGGTAGAAGGCTTCTTAA, GAAGTTCCTGAGCAAGTTATT	4	47, 55, 58, 51	1.2658	22	0.988	20
Sfmbt2	TTCGTCAACCACCGGTGTTTC, CGGATGTGGTACGATTCATTA, CCTATTTGATAGTCTATATT, CCCTCTGACCACACCATATAA	4	66, 49, 69, 65	1.5443	24	1	21
Egr2	CCACTCTCTACCATCCGTAAT, GAGATGGCATGATCAACATTG	2	71, 59	1.2143	20	1.0001	22
Kdm6a	GCTACGAATCTCTAATCTTAA, AGTTAGCAGTGGAAACGTTATG, CTATGCCAGGACTCTCGTAAA	3	67, 70, 73	1.4813	23	1.0001	23
Adam1a	GCACACAGTGTGATAGGATTT, TTCGCCAACATGTACGCTTAA	2	45, 76	1.2188	21	1.0001	24

[00145] Table 12. RIGER-assigned p values for depletion in the SLIC screen at 6 weeks.

Gene	Hairpins	# Hairpins	Hairpin ranks	Normalized enrichment score	Gene rank	p value	p value rank
Gpx6	GCATGTGCAATCTACAGAGAT, GTGAACGGAGACAATGAACAA, AGCCATTCAACGTCACGGTTT	3	19, 6, 7	0.1444	1	0.0321	1
Kdm3a	CACGATCAGAGCTGGTATTTA, TGCGGGTAGAAGGCTTCTTAA, CTGCGAAGTTTCGTTGGATTT, GAAGTTCCCTGAGCAAGTTATT	4	11, 9, 2, 45	0.1835	2	0.0655	2
Pcdhb8	CTGGCTCCAATGGCCTTATTA, CACAGATATAAACGACCATTT, AGACTTGCAGTTCACAGATAT	3	5, 16, 53	0.2834	3	0.1244	3
Taf13	AGAATTGAAACGGGCTAGAAA, CGAAGACCTTGTACATAGAGTT	2	13, 24	0.3795	4	0.1327	4
Adam1a	GCACACAGTGTGATAGGATTT, TTCGCCAACATGTACGCTTAA	2	21, 40	0.6295	5	0.3669	5
Phyh	GAGGACATCAAAGCAAAGAAA, GCTCTTCCTTATAATTCCTTT	2	25, 41	0.6607	7	0.4065	6
Pkp2	GCCTTGAGAACTTGGTATTT, CCTGAGTATGTCTACAAGCTA	2	27, 42	0.683	9	0.4366	7
Pea15a	CAAAGACAACCTCTCCTACAT, CCTGACCAACAACATCACCCCT, ACACCAAGCTAACCCGTATTC	3	30, 74, 29	0.6364	6	0.5145	8
Hras1	CGGGTGAAAGATTCAGATGAT, GTGAGATTCGGCAGCATAAAT, CACGTTGCATCACAGTAAATT	3	72, 34, 23	0.6684	8	0.5534	9
Ddit4l	GATTTGACTACTGGGATTAT, TCGCTTCTCCTCAGGCCTTAA, CCCTAATGAGTGGATAATAAAA	3	36, 20, 65	0.6845	10	0.5719	10
GFP	ACAACAGCCACAACGTCTATA, GCGATCACATGGTCCTGCTGG	2	50, 35	0.8259	11	0.6461	11
Gpr149	CCCCTTTCTTCTAGTTATAT, CCAGTGTGTTGTCTTATCCAAA, GCGATATTAACATATGGAGAAA	3	46, 54, 18	0.8342	12	0.7527	12
Igfbp4	CATTCCAAACTGTGACCGCAA, GACAAGGATGAGAGCGAACAT, GCTGCGGTTGTTGCGCCACTT	3	61, 48, 15	0.8503	13	0.7693	13
Foxp2	CGGAAGTTATTGATGTGGTAT, CGGACAGTCTTCAGTTCTGAA, GCGACATTCAGACAAATACAA	3	55, 38, 43	0.893	14	0.8132	14
Stk3	CCTGAGGTAATTCAAGAAATA, CCTTCTTTCATGGACTACTTT	2	4, 70	0.9554	15	0.8491	15
Pcdhb6	CCAGAATGCTTGGCTGTCATT, GCTCACACTCTACCTGGTCAT, CAAATTCCTGAACCATTATTC	3	10, 59, 60	1	16	0.9038	16
Egr2	CCACTCTTACCATCCGTAAT, GAGATGGCATGATCAACATTG	2	64, 39	1.0312	17	0.9305	17
B2m	GCCGAACATACTGAACTGCTA, CCAGTTTCTAATATGCTATAC, TAAAGTAGAGATGTCAGATAT	3	67, 63, 8	1.0535	18	0.9334	18

Sfmbt2	TTCGTCAACCACCGGTGTTTC, CGGATGTGGTACGATTCATTA, CCCTCTGACCACACCATATAA, CCTATTTGATAGTCCTATATT	4	37, 49, 51, 44	1.0696	20	0.934 2	19
Pedhbi4	CATGATACTGGTAGTCATATT, TCAGTACTTATCAGCGAAATT, GTAGTGCAACCATCACGTATT, AGGCAAGTGACCGCCATTATC	4	75, 69, 52, 14	1.0759	21	0.937 3	20
Arnt2	CGCTATTATCATGCCATAGAT, CCTACTCTGATGAGATCGAGT, TGTCGGACAAGGCAGTAAATA	3	28, 66, 58	1.0802	22	0.947 4	21
LUCIFERAS E	AGAATCGTCGTATGCAGTGAA, CACTCGGATATTTGATATGTG	2	17, 73	1.0536	19	0.949	22
Dpp10	GCTTCTTTATGAGCCAAATA, GGCATCCAGTGTACTGCATAA	2	71, 31	1.0893	23	0.972 6	23
Kdm6a	GCTACGAATCTCTAATCTTAA, AGTTAGCAGTGGAACGTTATG, CTATGCCAGGACTCTCGTAAA	3	57, 68, 56	1.2139	24	0.989 2	24

\* \* \*

[00146] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.



## WHAT IS CLAIMED IS:

1. A method of screening for modulators of a disease comprising:
  - (a) administering to each of a first and second mammal of the same species at least one vector, each vector comprising a regulatory element operably linked to a nucleotide sequence that is transcribed in vivo,
    - wherein the first mammal is a model of a human disease and the second mammal is a normal control mammal not a model of a human disease, and
    - wherein the nucleotide sequence encodes a protein coding gene, or a short hairpin RNA, or a CRISPR/Cas system;
  - (b) harvesting DNA from the first mammal and the second mammal;
  - (c) identifying the vectors by sequencing the harvested DNA; and
  - (d) comparing the representation of each vector from the first mammal and the second mammal, whereby a differential representation in the first mammal indicates that the protein coding gene, or short hairpin RNA target, or CRISPR/Cas system target is a modulator of the disease.
2. The method of claim 1, wherein each vector comprises a unique barcode sequence, and the method further comprises identifying the barcodes during sequencing, whereby the identification of a barcode indicates the presence of a vector.
3. The method of claim 1 or 2, wherein the vectors are administered stereotaxically.
4. The method of any of claims 1 to 3, wherein the CRISPR/Cas system comprises:
  - (i) a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence comprising at least one guide sequence, a tracr RNA, and a tracr mate sequence, wherein the at least one guide sequence hybridizes with a target sequence; and
  - (ii) a second regulatory element operably linked to a nucleotide sequence encoding a Type II Cas9 protein.
5. The method of any of claims 1 to 3, wherein the first and second mammals are transgenic non-human mammals comprising Cas9 and wherein the nucleotide sequence encoding a CRISPR/Cas system comprises at least one guide sequence, a tracr RNA, and a tracr mate sequence, wherein the at least one guide sequence hybridizes with a target sequence.

6. The method of claim 5, wherein expression of Cas9 is inducible.
7. The method of any of the preceding claims, wherein the vector is configured to be conditional, whereby the vector targets only certain cell types.
8. The method of any of the preceding claims, wherein the vector is a viral vector.
9. The method of claim 8, wherein the viral vector is a lentivirus, an adenovirus, or an adeno associated virus (AAV).
10. The method of any of the preceding claims, wherein the disease is Huntington's Disease.
11. The method of any of the preceding claims, wherein the first mammal is the R6/2 Huntington's disease model line.
12. A method of treating a nervous system disease comprising activating expression of *Gpx6* in the central nervous system of a subject in need thereof suffering from the disease.
13. A method of treating a nervous system disease comprising expressing *Gpx6* in the central nervous system of a subject in need thereof suffering from the disease.
14. A method of treating a nervous system disease comprising introducing into a subject in need thereof suffering from the disease a CRISPR-Cas9 based system configured to target *Gpx6*.
15. The method of claim 14, wherein the CRISPR/Cas system comprises a functional domain that activates transcription of the *Gpx6* gene.
16. The method of any of claims 12 to 15, wherein the nervous system disease is Huntington's Disease or Parkinson's Disease.
17. The method of any of claims 12, to 16, further comprising administering to a subject in need thereof suffering from the disease at least one of the drugs selected from the group consisting of Tetrabenazine, neuroleptics, benzodiazepines, amantadine, anti Parkinson's drugs, valproic acid, antioxidants, and Gpx mimetics.
18. A method of determining a prognosis for a central nervous system disease comprising:
  - (a) obtaining a RNA sample from a patient suffering from a central nervous system disease;
  - (b) assaying the level of *Gpx6* gene expression; and

(c) comparing the levels of *Gpx6* gene expression to a control level determined by testing healthy subjects, wherein the prognosis is worse if *Gpx6* gene expression is lower than the control level.

19. The method of claim 17 further comprising assaying the level of *DARPP-32* gene expression; and comparing the levels of *DARPP-32* gene expression to a control level determined by testing healthy subjects, wherein the prognosis is worse if *DARPP-32* gene expression is lower than the control level.

20. An antibody comprising a heavy chain and a light chain, wherein the antibody binds to an antigenic region of the *Gpx6* protein comprising SEQ ID No: 1.

Figure 1

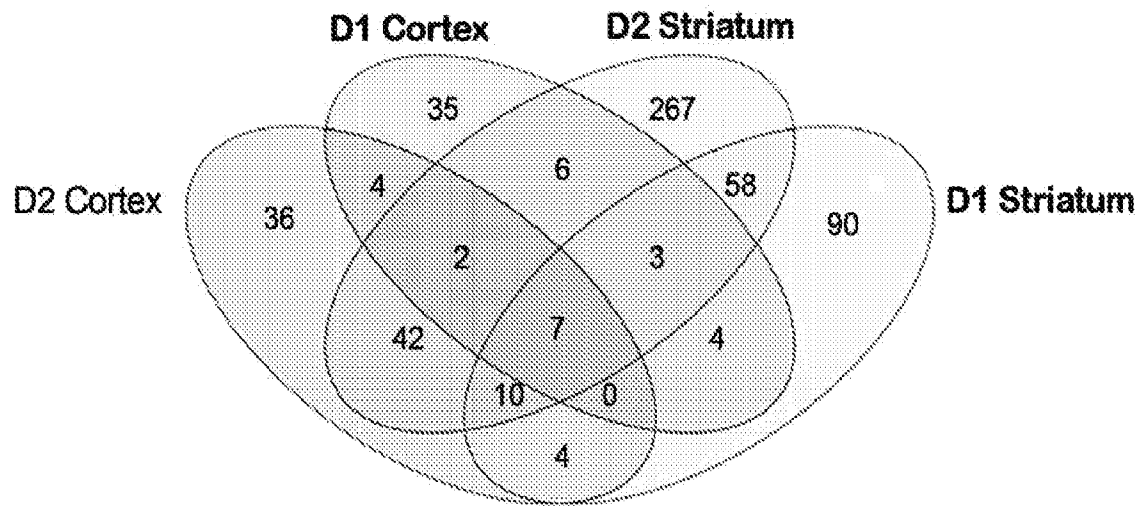


Figure 2

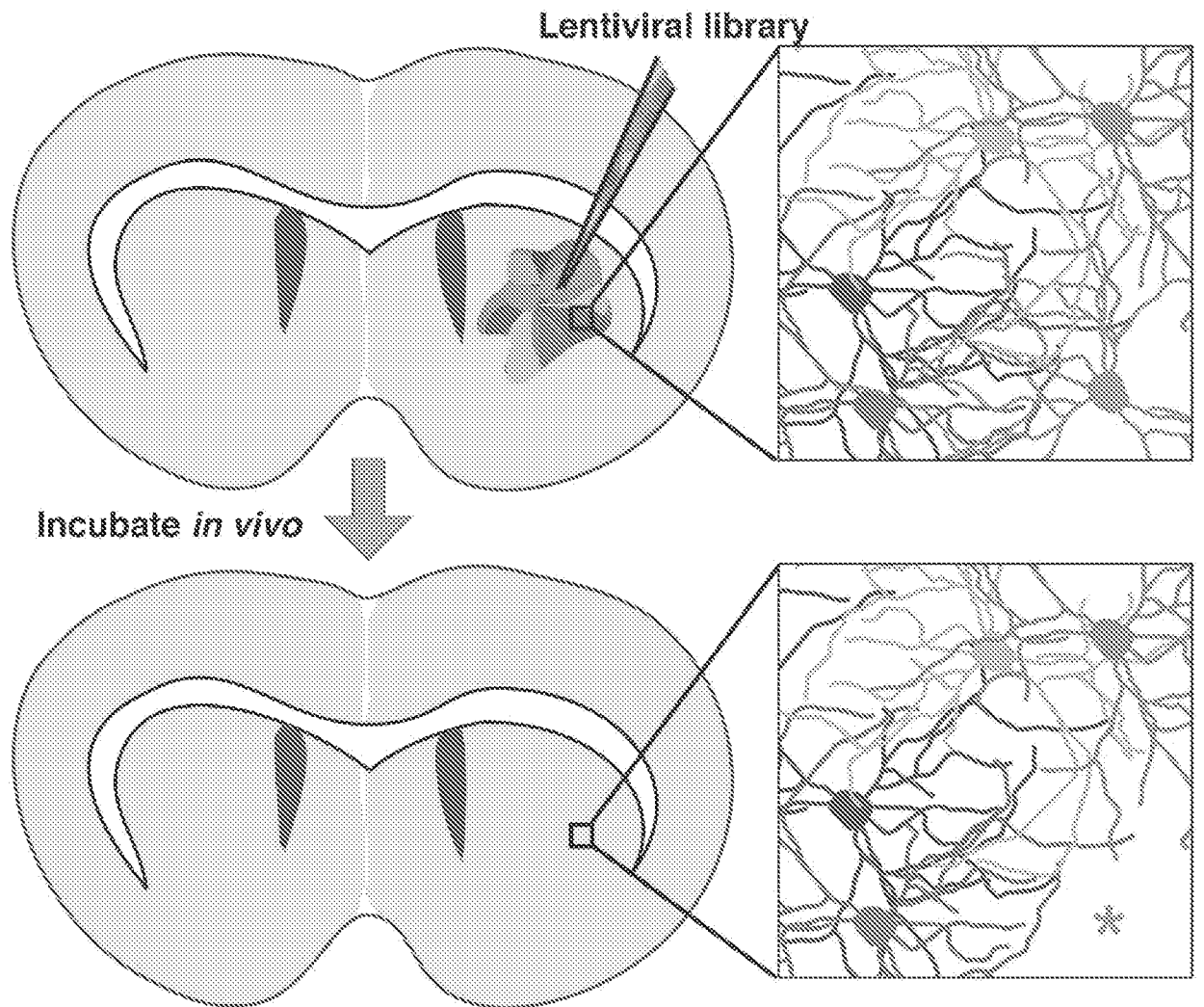


Figure 3

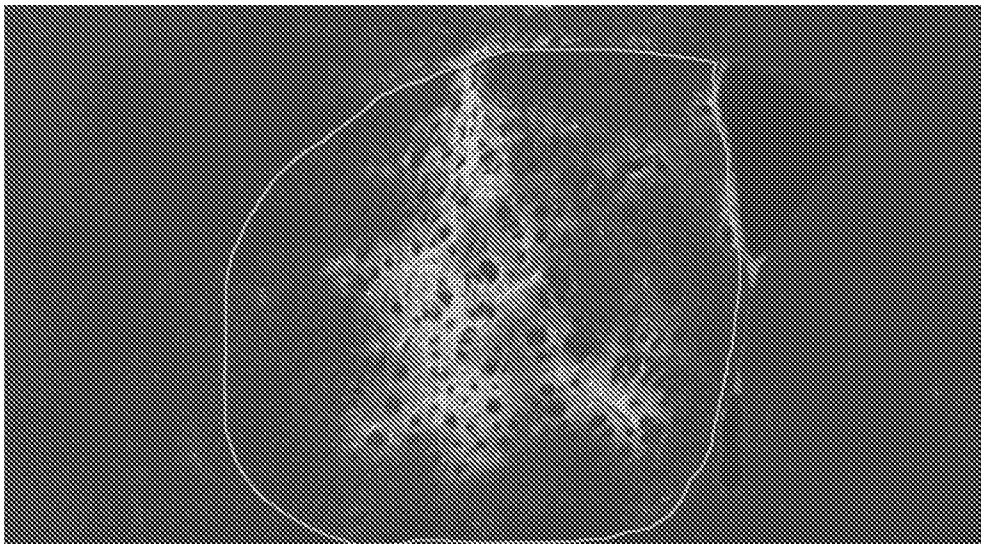
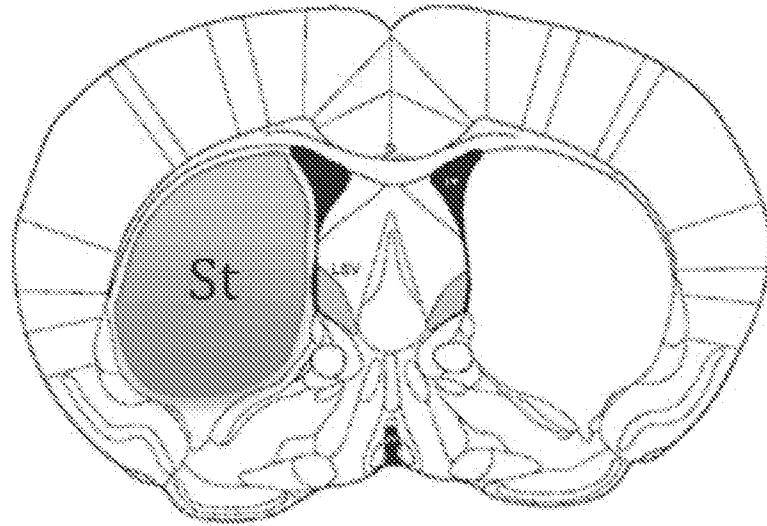
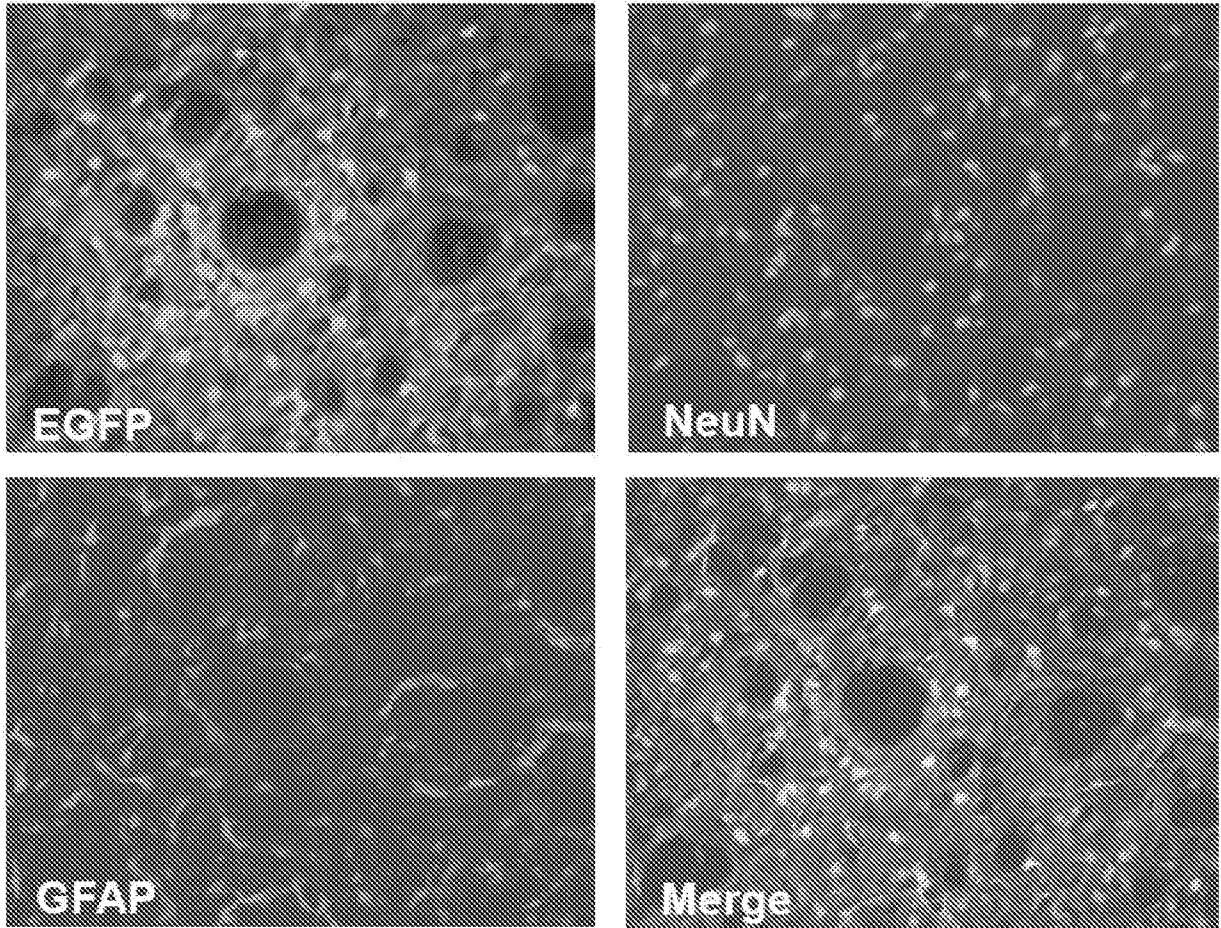


Figure 4



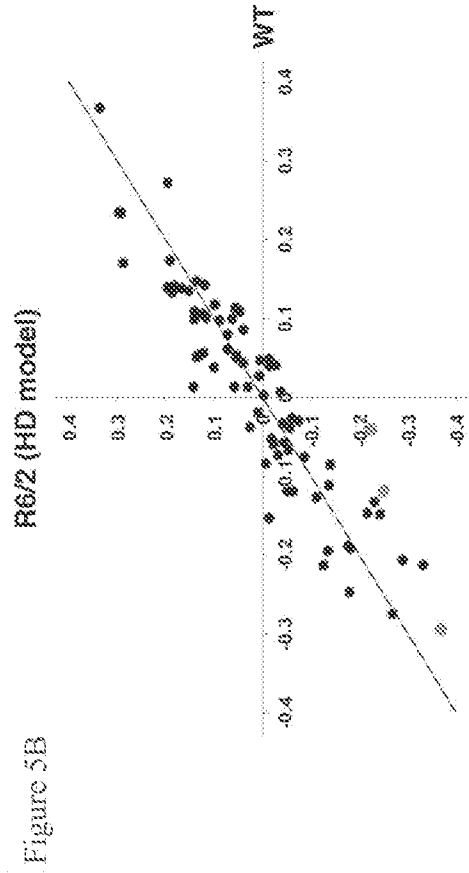


Figure 5B

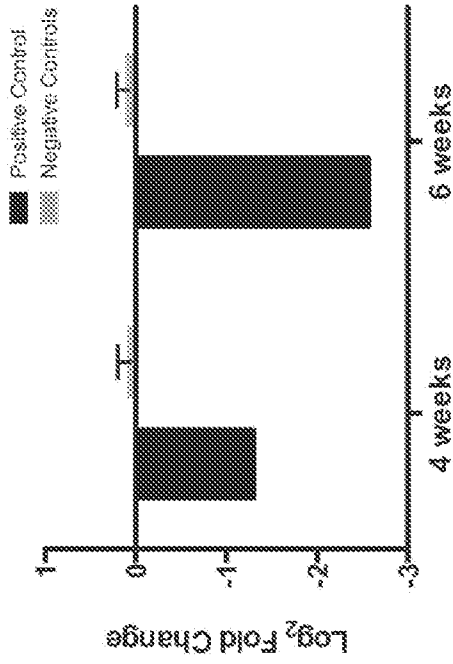
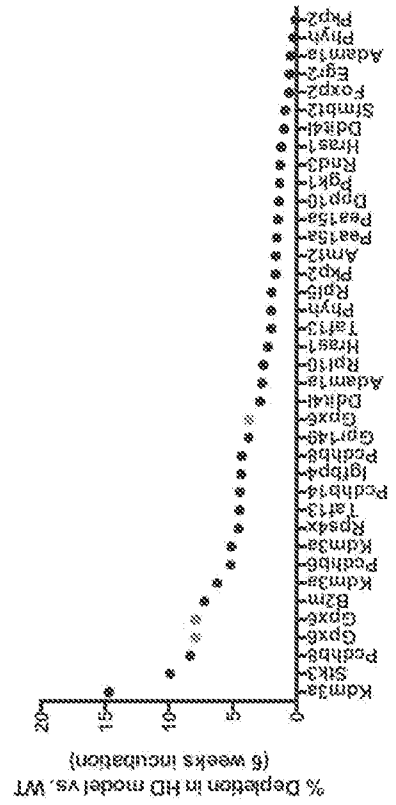


Figure 5A

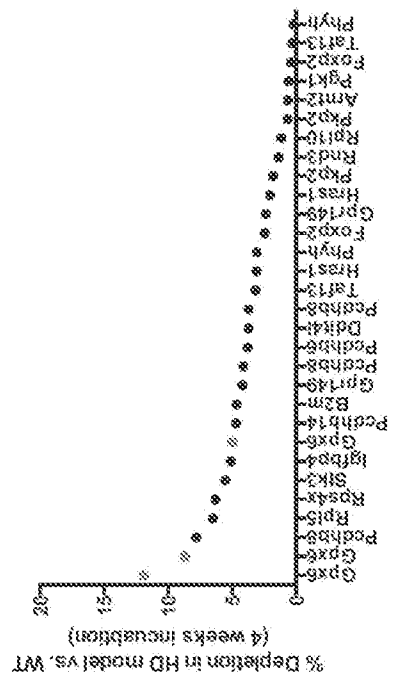


Figure 5C



Figure 6

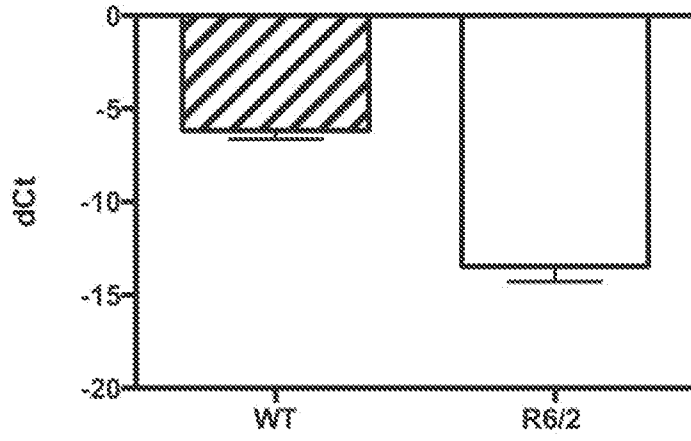


Figure 7

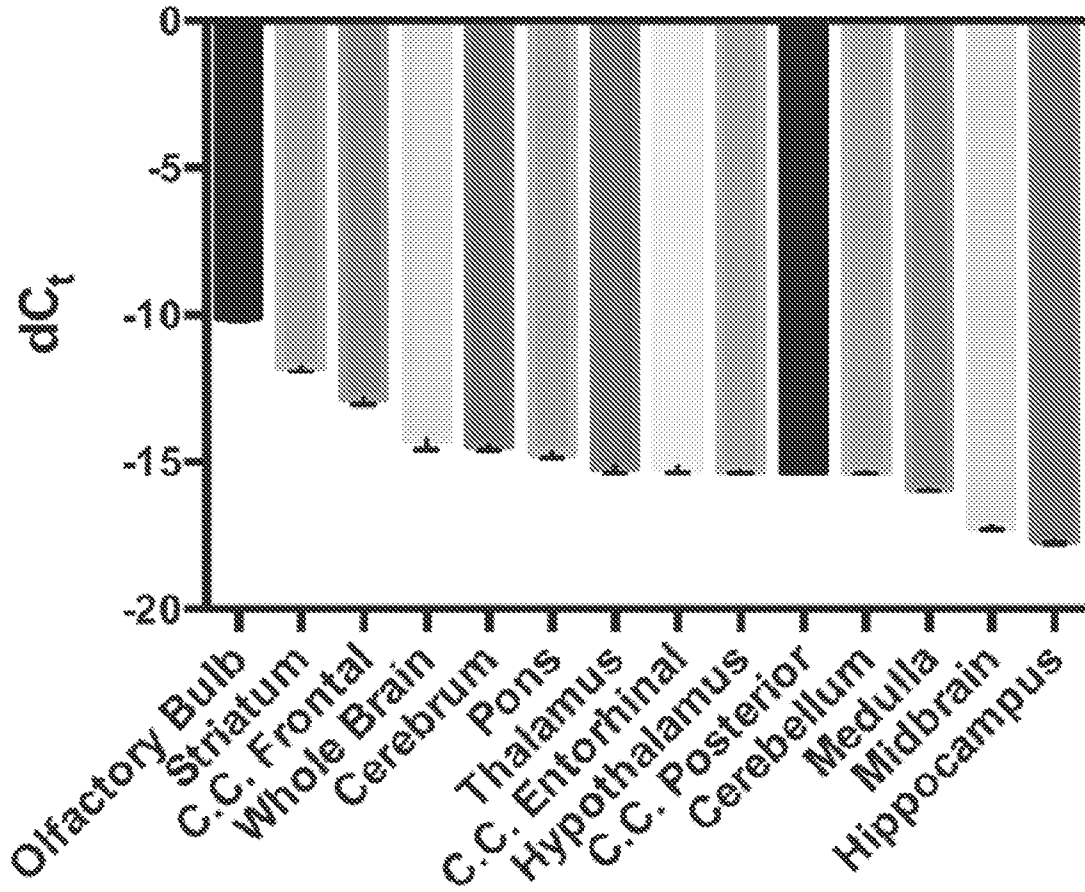


Figure 8

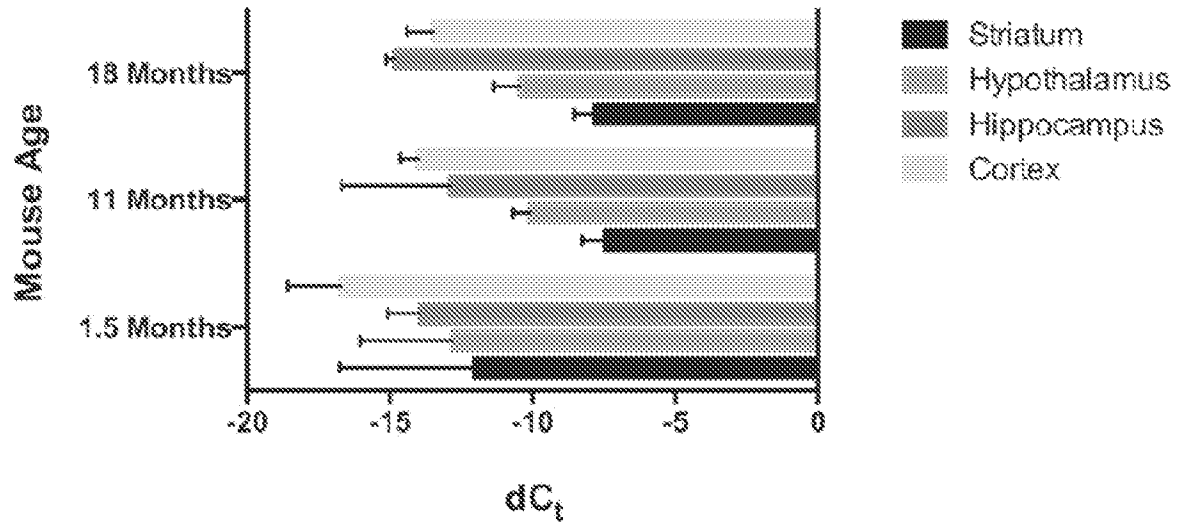


Figure 9A

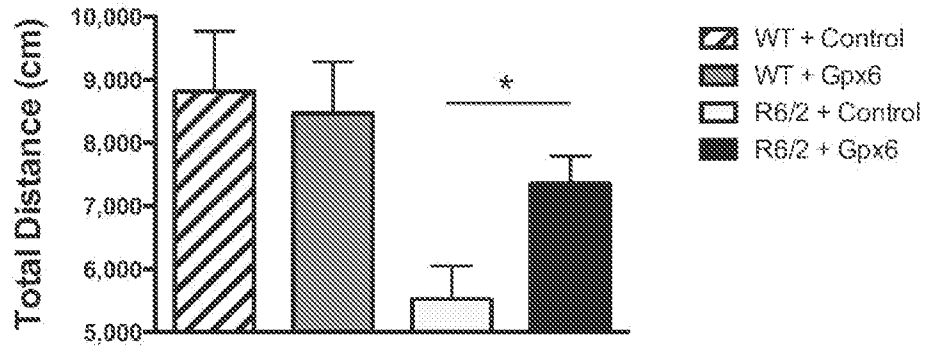


Figure 9B

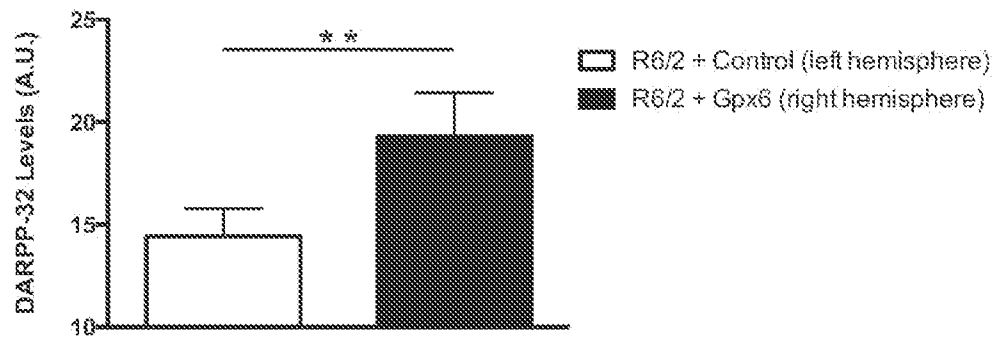
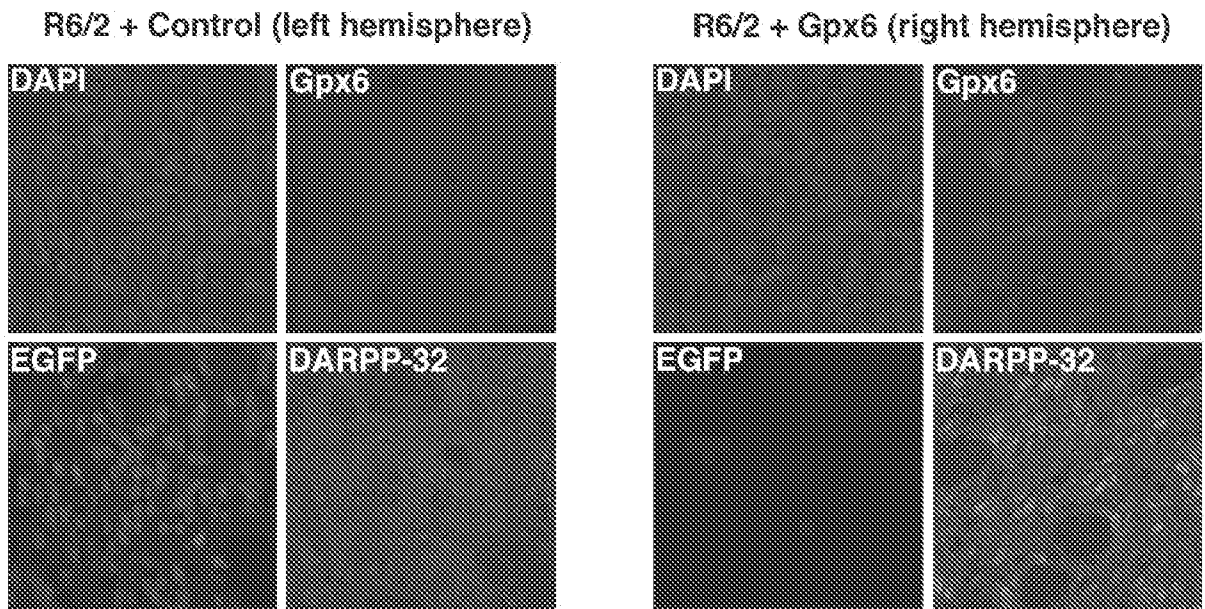


Figure 10

