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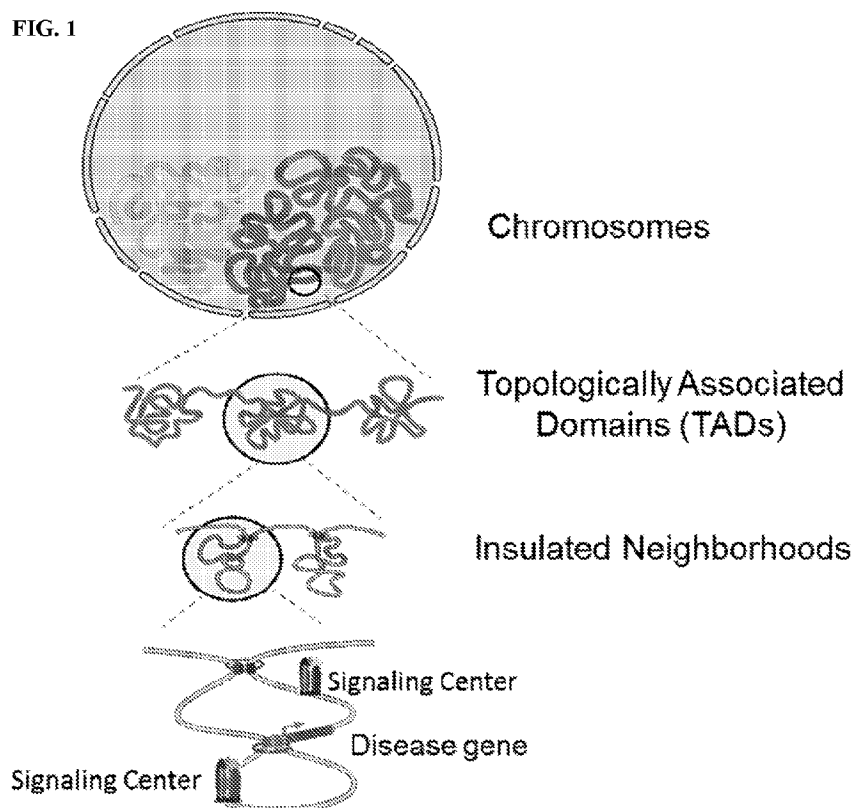
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(54) Titre : METHODES ET COMPOSITIONS POUR LE TRAITEMENT DE TROUBLES DU CYCLE DE L'UREE, EN PARTICULIER D'UNE DEFICIENCE EN OTC
(54) Title: METHODS AND COMPOSITIONS FOR TREATING UREA CYCLE DISORDERS, IN PARTICULAR OTC DEFICIENCY

FIG. 1



(57) **Abrégé/Abstract:**

The present invention provides methods and compositions for the treating a patient with a urea cycle disorder. Methods and compositions are also provided for modulating genes encoding enzymes that participate in the urea cycle by altering gene signaling networks.

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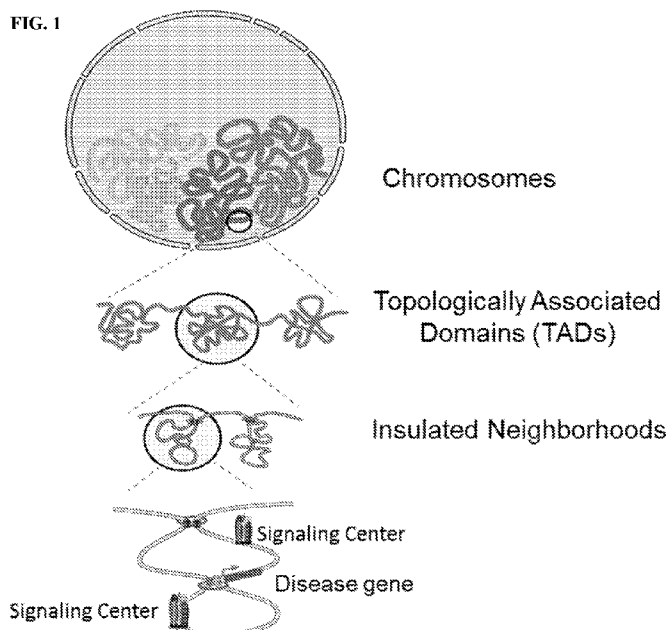
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FIG. 1



(57) Abstract: The present invention provides methods and compositions for the treating a patient with a urea cycle disorder. Methods and compositions are also provided for modulating genes encoding enzymes that participate in the urea cycle by altering gene signaling networks.

[Continued on next page]



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METHODS AND COMPOSITIONS FOR TREATING UREA CYCLE DISORDERS, IN PARTICULAR OTC DEFICIENCY

RELATED APPLICATION DATA

[001] This application claims the benefit of US Provisional Application Serial Number 62/568,893, filed October 6, 2017, the entire disclosure of which is incorporated for reference for all purposes.

SEQUENCE LISTING

[002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 20, 2018, is named 41744WO_CRF_sequencelisting.txt, and is 1,309,316 bytes in size.

FIELD OF THE INVENTION

[003] The present invention provides compositions and methods for the treatment of urea cycle disorders in humans.

BACKGROUND OF THE INVENTION

[004] Urea cycle disorders are a group of genetic disorders caused by defects in the metabolism of waste nitrogen via the urea cycle. The urea cycle is a cycle of biochemical reactions that produces urea from ammonia, a product of protein catabolism. The urea cycle mainly occurs in the mitochondria of liver cells. The urea produced by the liver enters the bloodstream where it travels to the kidneys and is ultimately excreted in urine. Genetic defects in any of the enzymes or transporters in the urea cycle can cause hyperammonemia (elevated blood ammonia), or the buildup of a cycle intermediate. Ammonia then reaches the brain through the blood, where it can cause cerebral edema, seizures, coma, long term disabilities in survivors, and/or death.

[005] The onset and severity of urea cycle disorders is highly variable. It is influenced by the position of the defective protein in the cycle and the severity of the defect. Mutations that lead to severe deficiency or total absence of activity of any of the enzymes can result in the accumulation of ammonia and other precursor metabolites during the first few days of life. Because the urea cycle is the principal clearance system for ammonia, complete disruption of this pathway results in the rapid accumulation of ammonia and development of related symptoms. Mild to moderate mutations represent a broad spectrum of enzyme function, providing some ability to detoxify ammonia, and result in mild to moderate urea cycle disorders.

[006] According to National Urea Cycle Disorders Foundation, the incidence of urea cycle disorders is estimated to be 1 in 8,500 live birth in the United States. The estimated incidence of individual urea cycle disorder varies from less than 1:2,000,000 to about 1:56,500 (See NA Mew

et al., Urea Cycle Disorders Overview, 2015). They occur in both children and adults. These disorders are most often diagnosed in infancy, but some children do not develop symptoms until early childhood. Newborns with severe urea cycle disorders become catastrophically ill within 36-48 hours of life. In children with mild or moderate urea cycle disorders, symptoms may be seen as early as one year of age. Early symptoms include disliking meat or other high-protein foods, inconsolable crying, failure to thrive, mental confusion, and hyperactive behavior. Symptoms can progress to frequent episodes of vomiting, lethargy, delirium, and coma. Some individuals with mild urea cycle defects are diagnosed in adulthood. Ammonia accumulation may be triggered by illness or stress (e.g., viral infection, surgery, prolonged fasting, excessive exercising, and excessive dieting), resulting in multiple mild elevations of plasma ammonia concentration. Without proper diagnosis and treatment, these individuals are at risk for permanent brain damage, coma, and death.

[007] Treatment for urea cycle disorders is a lifelong process. Symptoms are usually managed by using a combination of strategies including diet restriction, amino acid supplements, medications, dialysis, and/or hemofiltration. Dietary management is key to restricting the level of ammonia produced in the body. A careful balance of dietary protein, carbohydrates and fats is necessary to lower protein intake, while providing adequate calories for energy needs, as well as adequate essential amino acids for cell growth and development. Depending on the type of urea cycle disorder, amino acid supplements such as arginine or citrulline may be added to the diet. Sodium phenylbutyrate (BUPHENYL®), glycerol phenylbutyrate (RAVICTI®) and sodium benzoate are FDA approved drugs for the treatment of urea cycle disorders. They function as nitrogen binding agents to allow the kidneys to excrete excess nitrogen in place of urea. Dialysis and/or hemofiltration are used to quickly reduce plasma ammonia concentration to normal physiological level. When other treatment and management options fail, or for neonatal onset CPS1 and OTC deficiency, liver transplant is an option. Although the transplant alternative has been proven to be effective, the cost of the surgery, shortage of donors, and possible side effects of immunosuppressants can be difficult to overcome.

[008] Therefore, there is a high unmet need for developing effective therapeutics for the treatment of urea cycle disorders.

SUMMARY OF THE INVENTION

[009] The invention provides, among other things, methods of treating a subject with a urea cycle disorder. The methods include administering to the subject an effective amount of a compound capable of modulating the expression of one or more genes selected from Carbamoyl Phosphate Synthetase 1 (CPS1), Ornithine Transcarbamoylase (OTC), Argininosuccinate Synthetase 1 (ASS1), Argininosuccinate Lyase (ASL), N-Acetylglutamate Synthetase (NAGS),

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Arginase 1 (ARG1), Solute Carrier Family 25 Member 15 (SLC25A15), and Solute Carrier Family 25 Member 13 (SLC25A13). Such compound may be a small molecule, a polypeptide, an antibody, a hybridizing oligonucleotide, or a genome editing agent. In some embodiments, such compound may include at least one selected from Tables 2-10, or a derivative or an analog thereof.

[0010] In some embodiments, the invention provides methods for increasing OTC expression in a cell harboring an OTC mutation associated with a partial reduction of OTC function by contacting the cell with an effective amount of a compound that inhibits a target selected from the group consisting of JAK1, JAK2, JAK3, HSP90, MAPK, EGFR, FGFR, BRAF, RAF1, KDR, FLT1, TBK1, IKBKE, PRKAA1, PRKAA2, PRKAB1, BMPR1A and BMPR1B. In some embodiments the cell is a hepatocyte. In some embodiments the target is JAK1, JAK2 or JAK3 and the compound selected from the group consisting of Momelotinib and Baricitinib. In some embodiments the target is HSP90 and the compound is selected from the group consisting of 17-AAG, BIIB021, HSP-990, and Retaspimycin HCl. In some embodiments, the target is MAPK and the compound is selected from the group consisting of BIRB796, Pamapimod and PH-797804. In some embodiments, the target is EGFR and the compound is Mubritinib (TAK 165). In some embodiments, the target is FGFR and the compound is XL228. In some embodiments, the target is BRAF or RAF1 and the compound is selected from the group consisting of Lifirafenib (BGB-283) and BMS-214662. In some embodiments, the target is KDR or FLT1 and the compound is Foretinib/XL880 (GSK1363089). In some embodiments, the target is TBK1 or IKBKE and the compound is BX795. In some embodiments the target is PRKAA1, PRKAA2, or PRKAB1 and the compound is Dorsomorphin. In some embodiments, the OTC mutation is a mutation selected from the list of mutations in Table 26 that are associated with non-zero residual OTC function.

[0011] In some embodiments, the invention provides methods for increasing OTC expression in a human subject harboring an OTC mutation associated with a partial reduction of OTC function by administering to the subject an effective amount of a compound that inhibits a target selected from the group consisting of JAK1, JAK2, JAK3, HSP90, MAPK, EGFR, FGFR, BRAF, RAF1, KDR, FLT1, TBK1, IKBKE, PRKAA1, PRKAA2, PRKAB1, BMPR1A and BMPR1B. In some embodiments the target is JAK1, JAK2 or JAK3 and the compound selected from the group consisting of Momelotinib and Baricitinib. In some embodiments the target is HSP90 and the compound is selected from the group consisting of 17-AAG, BIIB021, HSP-990, and Retaspimycin HCl. In some embodiments, the target is MAPK and the compound is selected from the group consisting of BIRB796, Pamapimod and PH-797804. In some embodiments, the target is EGFR and the compound is Mubritinib (TAK 165). In some embodiments, the target is

FGFR and the compound is XL228. In some embodiments, the target is BRAF or RAF1 and the compound is selected from the group consisting of Lifirafenib (BGB-283) and BMS-214662. In some embodiments, the target is KDR or FLT1 and the compound is Foretinib/XL880 (GSK1363089). In some embodiments, the target is TBK1 or IKBKE and the compound is BX795. In some embodiments the target is PRKAA1, PRKAA2, or PRKAB1 and the compound is Dorsomorphin. In some embodiments, the OTC mutation is a mutation selected from the list of mutations in Table 20 that are associated with non-zero residual OTC function.

[0012] In some embodiments, the compound may be capable of modulating the expression of CPS1 and is at least one selected from Table 2, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of OTC and is at least one selected from Table 3, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ASS1 and is at least one selected from Table 4, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ASL and is at least one selected from Table 5, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of NAGS and is at least one selected from Table 6, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ARG1 and is at least one selected from Table 7, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of SLC25A15 and is at least one selected from Table 8, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of SLC25A13 and wherein the compound is at least one selected from Table 9, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of three or more genes selected from the group consisting of CPS1, OTC, ASS1, ASL, NAGS, ARG1, SLC25A15, and SLC25A13, and is at least one selected from Table 10, or a derivative or an analog thereof.

[0013] In some embodiments, the compound may increase the expression of the target genes. In some embodiments, the expression of the target genes may be increased by at least about 40%. In some embodiments, the expression of the target genes may be increased in the liver of the subject. In some embodiments, the subject may have at least one mutation within or near the target genes.

[0014] In some embodiments, the urea cycle disorder may be Carbamoyl Phosphate Synthetase 1 (CPS1) deficiency. In some embodiments, the urea cycle disorder may be Ornithine Transcarbamylase (OTC) deficiency. In some embodiments, the urea cycle disorder may be Argininosuccinate Synthetase (ASS1) deficiency. In some embodiments, the urea cycle disorder

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may be Argininosuccinate Lyase (ASL) deficiency. In some embodiments, the urea cycle disorder may be Arginase-1 (ARG1) deficiency. In some embodiments, the urea cycle disorder may be N-Acetylglutamate Synthetase (NAGS) deficiency. In some embodiments, the urea cycle disorder may be Ornithine translocase (ORNT1) deficiency. In some embodiments, the urea cycle disorder may be Citrin deficiency.

[0015] Also provided herein is a method of modulating the expression of one or more urea cycle-related genes in a cell. The method includes introducing into the cell an effective amount of a compound capable of altering one or more signaling molecules associated with the regulatory sequence regions (RSRs) or portion thereof of the urea cycle-related genes. The urea cycle-related genes may be one or more selected from CPS1, OTC, ASS1, ASL, NAGS, ARG1, SLC25A13, and SLC25A15. The compound may be a small molecule, a polypeptide, an antibody, a hybridizing oligonucleotide, or a genome editing agent. In some embodiments, such compound may include at least one selected from Tables 2-10, or a derivative or an analog thereof.

[0016] In some embodiments, the compound may be capable of modulating the expression of CPS1 and is at least one selected from Table 2, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of OTC and is at least one selected from Table 3, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ASS1 and is at least one selected from Table 4, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ASL and is at least one selected from Table 5, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of NAGS and is at least one selected from Table 6, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of ARG1 and is at least one selected from Table 7, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of SLC25A15 and is at least one selected from Table 8, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of SLC25A13 and wherein the compound is at least one selected from Table 9, or a derivative or an analog thereof. In some embodiments, the compound may be capable of modulating the expression of three or more genes selected from the group consisting of CPS1, OTC, ASS1, ASL, NAGS, ARG1, SLC25A13, and SLC25A15, and is at least one selected from Table 10, or a derivative or an analog thereof.

[0017] In some embodiments, the compound increases the expression of the urea cycle-related genes. In some embodiments, the expression of the urea cycle-related genes is increased

by at least about 40%. In some embodiments, the cell has at least one mutation within or near the urea cycle-related genes. In some embodiments, the cell is a hepatocyte.

[0018] Also provided herein is a method of modulating the expression of one or more urea cycle-related genes in a cell, comprising introducing into the cell an effective amount of a compound that may be capable of modulating the Platelet-derived Growth Factor Receptor (PDGFR)-mediated signaling pathway. The urea cycle-related genes may be selected from CPS1, OTC, ASS1, ASL, NAGS, ARG1, SLC25A13, and SLC25A15. The compound may be a small molecule, a polypeptide, an antibody, a hybridizing oligonucleotide, or a genome editing agent.

[0019] In some embodiments, the compound may be a PDGFR inhibitor. In some embodiments, the compound comprises CP-673451, or a derivative or an analog thereof. In some embodiments, the compound comprises Amuvatinib, or a derivative or an analog thereof. In some embodiments, the compound comprises Crenolanib, or a derivative or an analog thereof. In some embodiments, the compound may be a PDGFR activator. In some embodiments, the compound comprises PDGF, or a derivative or an analog thereof.

[0020] In some embodiments, the compound increases the expression of the urea cycle-related genes. In some embodiments, the expression of the urea cycle-related genes is increased by at least about 40%. In some embodiments, the cell has at least one mutation within or near the urea cycle-related genes. In some embodiments, the cell is a hepatocyte.

[0021] Further provided herein is a method of modulating the expression of one or more urea cycle-related genes in a cell. The method includes introducing into the cell an effective amount of a compound that may be capable of modulating the Transforming Growth Factor-beta (TGF-B) signaling pathway. The urea cycle-related genes may be selected from CPS1, OTC, ASS1, ASL, NAGS, ARG1, SLC25A13, and SLC25A15. The compound may be a small molecule, a polypeptide, an antibody, a hybridizing oligonucleotide, or a genome editing agent.

[0022] In some embodiments, the compound activates the TGF-B signaling pathway. In some embodiments, the compound comprises GDF2 (BMP9), or a derivative or an analog thereof. In some embodiments, the compound comprises BMP2, or a derivative or an analog thereof. In some embodiments, the compound comprises Activin, or a derivative or an analog thereof. In some embodiments, the compound comprises Nodal, or a derivative or an analog thereof. In some embodiments, the compound comprises Anti mullerian hormone, or a derivative or an analog thereof.

[0023] In some embodiments, the compound increases the expression of the urea cycle-related genes. In some embodiments, the expression of the urea cycle-related genes is increased by at least about 40%. In some embodiments, the cell has at least one mutation within or near the urea cycle-related genes. In some embodiments, the cell is a hepatocyte.

[0024] The present invention also provides a method of modulating the expression of a CPS1 gene in a cell. The method includes introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the CPS1 gene. In some embodiments, the upstream neighborhood genes include LANCL1-AS1 and LANCL1. In some embodiments, the downstream neighborhood genes include LOC107985978. In some embodiments, the cell has at least one mutation within or near the CPS1 gene. In some embodiments, the cell is a hepatocyte.

[0025] The present invention also provides a method of modulating the expression of an OTC gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the OTC gene. In some embodiments, the upstream neighborhood genes include RPGR. In some embodiments, the downstream neighborhood genes include LOC392442. In some embodiments, the cell has at least one mutation within or near the OTC gene. In some embodiments, the cell is a hepatocyte.

[0026] The present invention also provides a method of modulating the expression of an ASS1 gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the ASS1 gene. In some embodiments, the upstream neighborhood genes include HMCN2 and LOC107987134. In some embodiments, the downstream neighborhood genes include FUBP3 and LOC100272217. In some embodiments, the cell has at least one mutation within or near the ASS1 gene. In some embodiments, the cell is a hepatocyte.

[0027] The present invention also provides a method of modulating the expression of an ASL gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the ASL gene. In some embodiments, the upstream neighborhood genes include LOC644667. In some embodiments, the downstream neighborhood gene include CRCP. In some embodiments, the cell has at least one mutation within or near the ASL gene. In some embodiments, the cell is a hepatocyte.

[0028] The present invention also provides a method of modulating the expression of an NAGS gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the NAGS gene. In some embodiments, the upstream neighborhood genes include PPY. In some embodiments, the downstream neighborhood genes include TMEM101. In some embodiments, the cell has at least one mutation within or near the NAGS gene. In some embodiments, the cell is a hepatocyte.

[0029] The present invention also provides a method of modulating the expression of an ARG1 gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the ARG1 gene. In some embodiments, the upstream neighborhood genes include RPL21P67. In some embodiments, the downstream neighborhood genes include ENPP3. In some embodiments, the cell has at least one mutation within or near the ARG1 gene. In some embodiments, the cell is a hepatocyte.

[0030] The present invention also provides a method of modulating the expression of an SLC25A15 gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the SLC25A15 gene. In some embodiments, the upstream neighborhood genes include MRPS31. In some embodiments, the downstream neighborhood genes include MIR621. In some embodiments, the cell has at least one mutation within or near the SLC25A15 gene. In some embodiments, the cell is a hepatocyte.

[0031] The present invention also provides a method of modulating the expression of an SLC25A13 gene in a cell, comprising introducing to the cell one or more compounds that alter one or more of the upstream or downstream neighborhood genes or its RSRs of the insulated neighborhood comprising the SLC25A13 gene. In some embodiments, the upstream neighborhood genes include DYNC1I1. In some embodiments, the downstream neighborhood genes include RNU6-532P. In some embodiments, the cell has at least one mutation within or near the SLC25A13 gene. In some embodiments, the cell is a hepatocyte.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] FIG. 1 illustrates the packaging of chromosomes in a nucleus, the localized topological domains into which chromosomes are organized, insulated neighborhoods in TADs and finally an example of an arrangement of a signaling center(s) around a particular disease gene.

[0033] FIG. 2A and FIG. 2B illustrate a linear and 3D arrangement of the CTCF boundaries of an insulated neighborhood.

[0034] FIG. 3A and FIG. 3B illustrate tandem insulated neighborhoods and gene loops formed in such insulated neighborhoods.

[0035] FIG. 4 illustrates the concept of an insulated neighborhood contained within a larger insulated neighborhood and the signaling which may occur in each.

[0036] FIG. 5 illustrates the components of a signaling center; including transcriptional factors, signaling proteins, and/or chromatin regulators.

DETAILED DESCRIPTION OF THE INVENTION**I. INTRODUCTION**

[0037] The present invention provides compositions and methods for the treatment of urea cycle disorders in mammalian subjects, particularly in human subjects. In particular, the invention provides compounds and related use for the modulation of at least one gene encoding a protein (e.g., an enzyme or a transporter) involved in the urea cycle.

Binding sites for signaling molecules

[0038] A series of consensus binding sites, or binding motifs for binding sites, for signaling molecules has been identified by the present inventors. These consensus sequences reflect binding sites along a chromosome, gene, or polynucleotide for signaling molecules or for complexes which include one or more signaling molecules. These sites are provided by Table 11 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, and is reproduced below as Table 13 of the instant specification.

[0039] In some embodiments, binding sites are associated with more than one signaling molecule or complex of molecules. Further, non-limiting examples of such motifs or sites are also provided in Table 12 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, and is reproduced below as Table 14 of the instant specification.

[0040] It has further been determined that certain patterns are found in the binding motifs. A list of such patterns for complexes is provided by Tables 13 and 14 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, and for single molecules is provided in Tables 15 and 16 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety. Each of these are reproduced below, respectively, as Tables 15-18 of the instant specification.

[0041] In the Motif Tables 13-16 of U.S. 62/501,795 which is hereby incorporated by reference in its entirety, certain designators are used according to the IUPAC nucleotide code. This code is shown in Table 17 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, and is reproduced below as Table 19 of the instant specification.

[0042] Table 18 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, provides a list of signaling molecules including those which act as transcription factors (TF) and/or chromatin remodeling factors (CR) that function in various cellular signaling pathways. The methods described herein may be used to inhibit or activate the expression of one or more signaling molecules associated with the regulatory sequence region of the primary neighborhood gene encoded within an insulated neighborhood. The methods may thus alter the signaling signature of one or more primary neighborhood genes which are differentially expressed upon treatment with the therapeutic agent compared to an untreated control.

[0043] Various embodiments of the transcripts encoding the signaling proteins of Table 18 of U.S. 62/501,795, which is hereby incorporated by reference in its entirety, contain internal stop codons. These internal stop codons result in translation of multiple polypeptides. In one embodiment, a polypeptide that is a fragment of the signaling proteins taught in Table 18 of U.S. 62/501,795 may have signaling properties. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 9 and SEQ ID NO: 10 from SEQ ID NO: 11. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 12 and SEQ ID NO: 13 from SEQ ID NO: 14. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 15 and SEQ ID NO: 16 from SEQ ID NO: 17. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 18-19 from SEQ ID NO: 20. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 21 and SEQ ID NO: 22 from SEQ ID NO: 23. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 24 and SEQ ID NO: 25 from SEQ ID NO: 26. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 27 and SEQ ID NO: 28 from SEQ ID NO: 29. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 30 and SEQ ID NO: 31 from SEQ ID NO: 32. As a non-limiting example, the polypeptide may be a fragment such as SEQ ID NO: 33-37 from SEQ ID NO: 38.

[0044] In some embodiments, at least one compound selected from Tables 19-21, of U.S. 62/501,795, which are hereby incorporated by reference in their entirety, and Tables 22-26 and 28 of U.S. 62/501,795, which are hereby incorporated by reference in their entirety, may be used to modulate RNAs derived from regulatory sequence regions to alter or elucidate the gene signaling networks of the present invention.

II. UREA CYCLE DISORDERS AND RELATED GENES

[0045] Compositions and methods described herein may be used to treat one or more urea cycle disorders. As used herein, the term “urea cycle disorder” refers to any disorder that is caused by a defect or malfunction in the urea cycle. The urea cycle is a cycle of biochemical reactions that produces urea from ammonia, a product of protein catabolism. It is composed of 5 key enzymes including carbamoyl phosphate synthetase 1 (CPS1), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (ASS1), argininosuccinate lyase (ASL), and arginase 1 (ARG1), but also requires other enzymes, such as N-acetylglutamate synthetase (NAGS), and mitochondrial amino acid transporters, such as ornithine translocase (ORNT1) and citrin.

[0046] As used herein, a “urea cycle-related gene” refers to a gene whose gene product (e.g., RNA or protein) is involved in the urea cycle. Urea cycle-related genes include, but are not limited to, CPS1 (encoding CPS1), OTC (encoding OTC), ASS1 (encoding ASS1), NAGS (encoding NAGS), ARG1 (encoding ARG1), SLC25A15 (encoding ORNT1), and SLC25A13

(encoding citrin). Mutations in the urea cycle-related genes or their regulatory regions may lead to production of dysfunctional proteins and disruption of the urea cycle. In some cases, patients with a urea cycle disorder may carry a single functional allele and a mutated allele of a urea cycle-related gene. This results in the production of insufficient amount of the functional protein. The phenomenon is known as “haploinsufficiency.”

[0047] The urea cycle mainly occurs in the mitochondria of liver cells. The urea produced by the liver enters the bloodstream where it travels to the kidneys and is ultimately excreted in urine. Genetic defects in any of the enzymes or transporters in the urea cycle can cause hyperammonemia (elevated blood ammonia), or the buildup of a cycle intermediate. Ammonia then reaches the brain through the blood, where it can cause cerebral edema, seizures, coma, long term disabilities in survivors, and/or death.

[0048] The onset and severity of urea cycle disorders is highly variable. It is influenced by the position of the defective protein in the cycle and the severity of the defect. Mutations that lead to severe deficiency or total absence of activity of any of the first four enzymes in the pathway (CPS1, OTC, ASS1, and ASL) or the cofactor producer (NAGS) can result in the accumulation of ammonia and other precursor metabolites during the first few days of life. Because the urea cycle is the principal clearance system for ammonia, complete disruption of this pathway results in the rapid accumulation of ammonia and development of related symptoms. Mild to moderate mutations represent a broad spectrum of enzyme function, providing some ability to detoxify ammonia, and result in mild to moderate urea cycle disorders.

[0049] According to National Urea Cycle Disorders Foundation, the incidence of urea cycle disorders is estimated to be 1 in 8,500 live birth in the United States. The estimated incidence of individual urea cycle disorder varies from less than 1:2,000,000 to about 1:56,500 (See NA Mew et al., Urea Cycle Disorders Overview, 2015, which is incorporated by reference in its entirety). They occur in both children and adults. These disorders are most often diagnosed in infancy, but some children do not develop symptoms until early childhood. Newborns with severe urea cycle disorders become catastrophically ill within 36-48 hours of life. In children with mild or moderate urea cycle disorders, symptoms may be seen as early as one year of age. Early symptoms include disliking meat or other high-protein foods, inconsolable crying, failure to thrive, mental confusion or hyperactive behavior. Symptoms can progress to frequent episodes of vomiting, lethargy, delirium, and coma. Some individuals with mild urea cycle defects are diagnosed in adulthood. Ammonia accumulation may be triggered by illness or stress (e.g., viral infection, surgery, prolonged fasting, excessive exercising, and excessive dieting), resulting in multiple mild elevations of plasma ammonia concentration. Without proper diagnosis and treatment, these individuals are at risk for permanent brain damage, coma, and death.

[0050] Treatment for urea cycle disorders is a lifelong process. Symptoms are usually managed by using a combination of strategies including diet restriction, amino acid supplements, medications, dialysis, and/or hemofiltration. Dietary management is key to restricting the level of ammonia produced in the body. A careful balance of dietary protein, carbohydrates and fats is necessary to lower protein intake, while providing adequate calories for energy needs, as well as adequate essential amino acids for cell growth and development. Depending on the type of urea cycle disorder, amino acid supplements such as arginine or citrulline may be added to the diet. Sodium phenylbutyrate (BUPHENYL®), glycerol phenylbutyrate (RAVICTI®) and sodium benzoate are FDA approved drugs for the treatment of urea cycle disorders. They function as nitrogen binding agents to allow the kidneys to excrete excess nitrogen in place of urea. Dialysis and/or hemofiltration are used to quickly reduce plasma ammonia concentration to normal physiological level. When other treatment and management options fail, or for neonatal onset CPS1 and OTC deficiency, liver transplant is an option. Although the transplant alternative has been proven to be effective, the cost of the surgery, shortage of donors, and possible side effects of immunosuppressants can be difficult to overcome.

[0051] Specific types of urea cycle disorder include, but are not limited to, Phosphate Synthetase 1 (CPS1) deficiency, Ornithine Transcarbamylase (OTC) deficiency, Argininosuccinate Synthetase (ASS1) deficiency, Argininosuccinate Lyase (ASL) deficiency, Arginase-1 (ARG1) deficiency, N-Acetylglutamate Synthetase (NAGS) deficiency, Ornithine translocase (ORNT1) deficiency, and Citrin deficiency. Any one or more of these disorders may be treated or targeted by the compositions and methods described herein.

Carbamoyl Phosphate Synthetase 1 (CPS1) deficiency

[0052] In some embodiments, methods and compositions of the present invention may be used to treat Carbamoyl Phosphate Synthetase 1 (CPS1) deficiency. CPS1 deficiency (MIM #237300) is an autosomal recessive disorder caused by mutations in the CPS1 gene. CPS1 catalyzes the synthesis of carbamoyl phosphate from ammonia and bicarbonate. CPS1 deficiency is the most severe type of the urea cycle disorders. Approximately 10 mutations that cause CPS1 deficiency have been identified in the CPS1 gene. Individuals with complete CPS1 deficiency rapidly develop hyperammonemia in the newborn period. Children who are successfully rescued from crisis are chronically at risk for repeated episodes of hyperammonemia.

[0053] In some embodiments, methods of the present invention involve modulating the expression of the CPS1 gene. CPS1 may also be referred to as Carbamoyl-Phosphate Synthase 1, Mitochondrial; Carbamoyl-Phosphate Synthase (Ammonia); EC 6.3.4.16; Carbamoyl-Phosphate Synthase [Ammonia], Mitochondrial; Carbamoyl-Phosphate Synthetase I; Carbamoylphosphate Synthetase I; CPSase I; CPSASE1; and PHN. The CPS1 gene has a cytogenetic location of 2q34

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and the genomic coordinate are on Chromosome 2 on the forward strand at position 210,477,682-210,679,107. LANCL1-AS1 (ENSG00000234281) and LANCL1 (ENSG00000115365) are the genes upstream of CPS1 and LOC107985978 is the gene downstream of CPS1. CPS1-IT1 (ENSG00000280837) is a gene located within CPS1 on the forward strand. The CPS1 gene has a NCBI gene ID of 1373, Uniprot ID of P31327 and Ensembl Gene ID of ENSG00000021826. The genomic sequence of CPS1 is shown as in SEQ ID NO: 1.

Ornithine Transcarbamylase (OTC) deficiency

[0054] In some embodiments, methods and compositions of the present invention may be used to treat Ornithine Transcarbamylase (OTC) deficiency. OTC deficiency (MIM #311250) is an X-linked genetic disorder caused by mutations in the OTC gene. OTC catalyzes the reaction between carbamoyl phosphate and ornithine to form citrulline and phosphate. More than 500 OTC gene mutations have been identified in people with OTC deficiency. The severe, early-onset form of the disorder, caused by complete absence of OTC activity, usually affects males. This form is as severe as CPS1 deficiency. The later-onset form of the disorder occurs in both males and females. These individuals develop hyperammonemia during their lifetime and many require chronic medical management for hyperammonemia.

[0055] In some embodiments, methods of the present invention involve modulating the expression of the OTC gene. OTC may also be referred to as Ornithine Carbamoyltransferase; Ornithine Transcarbamylase; EC 2.1.3.3; OTCase, Ornithine Carbamoyltransferase, Mitochondrial; EC 2.1.3; and OCTD. The OTC gene has a cytogenetic location of Xp11.4 and the genomic coordinate are on Chromosome X on the forward strand at position 38,352,545-38,421,450. RPGR (ENSG00000156313) is the gene upstream of OTC and LOC392442 is the gene downstream of OTC. TDGF1P1 (ENSG00000227988) is the gene located within OTC on the reverse strand. The OTC gene has a NCBI gene ID of 5009, Uniprot ID of P00480 and Ensembl Gene ID of ENSG00000036473. The genomic sequence of OTC is shown as in SEQ ID NO: 2.

Argininosuccinate Synthetase (ASS1) deficiency

[0056] In some embodiments, methods and compositions of the present invention may be used to treat Argininosuccinate Synthetase (ASS1) deficiency. ASS1 deficiency (MIM #215700), also known as Citrullinemia type I, is an autosomal recessive disorder caused by mutations in the ASS1 gene. ASS1 catalyzes the synthesis of argininosuccinate from citrulline and aspartate. About 118 mutations that cause ASS1 deficiency have been identified in the ASS1 gene. The early onset form of this disorder can also be quite severe. The symptoms associated with hyperammonemia are life-threatening in many cases. Affected individuals are able to incorporate

some waste nitrogen into urea cycle intermediates, which makes treatment slightly easier than in the other urea cycle disorders.

[0057] In some embodiments, methods of the present invention involve modulating the expression of the ASS1 gene. ASS1 may also be referred to as EC 6.3.4.5, Argininosuccinate Synthase, ASS, Argininosuccinic Acid Synthetase 1, Argininosuccinate Synthetase 1, Argininosuccinate Synthetase, Citrulline-Aspartate Ligase, and CTLN1. The ASS1 gene has a cytogenetic location of 9q34.11 and the genomic coordinate are on Chromosome 9 on the forward strand at position 130,444,929-130,501,274. HMCN2 (ENSG00000148357) and LOC107987134 are the genes upstream of ASS1, and FUBP3 (ENSG00000107164) and LOC100272217 are the genes downstream of ASS1. LOC105376294 is the gene that overlaps with the 3' region of ASS1 on the reverse strand. The ASS1 gene has a NCBI gene ID of 445, Uniprot ID of P00966 and Ensembl Gene ID of ENSG00000130707. The genomic sequence of ASS1 is shown as in SEQ ID NO: 3.

Argininosuccinate Lyase (ASL) deficiency

[0058] In some embodiments, methods and compositions of the present invention may be used to treat Argininosuccinate Lyase (ASL) deficiency. ASL deficiency (MIM #207900) is an autosomal recessive disorder caused by mutations in the ASL gene. ASL cleaves argininosuccinic acid to produce arginine and fumarate in the fourth step of the urea cycle. More than 30 different mutations in the ASL gene have been identified worldwide. This disorder has a severe neonatal onset form and a late onset form. The severe neonatal onset form is indistinguishable from that of other urea cycle disorders. The late onset form ranges from episodic hyperammonemia triggered by acute infection or stress to cognitive impairment, behavioral abnormalities, and/or learning disabilities in the absence of any documented episodes of hyperammonemia.

[0059] In some embodiments, methods of the present invention involve modulating the expression of the ASL gene. ASL may also be referred to as Argininosuccinase, EC 4.3.2.1, ASAL, and Argininosuccinase. The ASL gene has a cytogenetic location of 7q11.21 and the genomic coordinate are on Chromosome 7 on the forward strand at position 66,075,798-66,093,558. LOC644667 is the gene upstream of ASL and CRCP (ENSG00000241258) is the genes downstream of ASL. The ASL gene has a NCBI gene ID of 435, Uniprot ID of P04424 and Ensembl Gene ID of ENSG00000126522. The genomic sequence of ASL is shown as in SEQ ID NO: 4.

N-Acetylglutamate Synthetase (NAGS) deficiency

[0060] In some embodiments, methods and compositions of the present invention may be used to treat N-Acetylglutamate Synthetase (NAGS) deficiency. NAGS deficiency (MIM

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#237310) is an autosomal recessive disorder caused by mutations in the NAGS gene. NAGS catalyzes the production of N-Acetylglutamate (NAG) from glutamate and acetyl-CoA. NAG is a cofactor of CPS1. Approximately 12 mutations in the NAGS gene have been identified in people with NAGS deficiency. Symptoms of NAGS deficiency mimic those of CPS1 deficiency, as CPS1 is rendered inactive in the absence of NAG.

[0061] In some embodiments, methods of the present invention involve modulating the expression of the NAGS gene. NAGS may also be referred to as Amino-Acid Acetyltransferase, N-Acetylglutamate Synthase, Mitochondrial, EC 2.3.1.1, AGAS, and ARGAs. The NAGS gene has a cytogenetic location of 17q21.31 and the genomic coordinate are on Chromosome 17 on the forward strand at position 44,004,546-44,009,063. PPY (ENSG00000108849) is the gene upstream of NAGS and TMEM101 (ENSG00000091947) is the genes downstream of NAGS. PYY (ENSG00000131096) is a gene that overlaps with NAGS on the reserve strand. The NAGS gene has a NCBI gene ID of 162417, Uniprot ID of Q8N159 and Ensembl Gene ID of ENSG00000161653. The genomic sequence of NAGS is shown as in SEQ ID NO: 5.

Arginase-1 (ARG1) deficiency

[0062] In some embodiments, methods and compositions of the present invention may be used to treat Arginase-1 (ARG1) deficiency. ARG1 deficiency (MIM #207800) is an autosomal recessive disorder caused by mutations in the ARG1 gene. ARG1 catalyzes the hydrolysis of arginine to ornithine and urea, which is the final step in the urea cycle. More than 40 mutations have been found in the ARG1 gene that cause partial or complete loss of enzyme function. Defects in ARG1 cause hyperargininemia, a more subtle disorder involving neurologic symptoms. Arginase deficiency usually becomes evident by about the age of 3. It most often appears as stiffness, especially in the legs, caused by abnormal tensing of the muscles (spasticity). Other symptoms may include slower than normal growth, developmental delay and eventual loss of developmental milestones, intellectual disability, seizures, tremor, and difficulty with balance and coordination (ataxia). Occasionally, high protein meals or stress caused by illness or periods without food (fasting) may cause ammonia to accumulate more quickly in the blood. This rapid increase in ammonia may lead to episodes of irritability, refusal to eat, and vomiting. In some affected individuals, signs and symptoms of arginase deficiency may be less severe, and may not appear until later in life. Hyperammonemia is rare or usually not severe in Arginase deficiency. Arginase deficiency is a very rare disorder; it has been estimated to occur once in every 300,000 to 1,000,000 individuals.

[0063] In some embodiments, methods of the present invention involve modulating the expression of the ARG1 gene. ARG1 may also be referred to as Liver-Type Arginase; Type I Arginase; Arginase, liver; and EC 3.5.3.1. The ARG1 gene has a cytogenetic location of 6q23.2

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and the genomic coordinate are on Chromosome 6 on the forward strand at position 131,573,144-131,584,332. RPL21P67 (ENSG00000219776) is the gene upstream of ARG1 and ENPP3 (ENSG00000154269) is the genes downstream of ARG1. MED23 (ENSG00000112282) is a gene that overlaps with ARG1 on the reserve strand. The ARG1 gene has a NCBI gene ID of 383, Uniprot ID of P05089 and Ensembl Gene ID of ENSG00000118520. The genomic sequence of ARG1 is shown as in SEQ ID NO: 6.

Ornithine translocase (ORNT1) deficiency

[0064] In some embodiments, methods and compositions of the present invention may be used to treat Ornithine translocase (ORNT1) deficiency. ORNT1 deficiency (MIM #238970), also known as the hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, is an autosomal recessive disorder caused by mutations in the SLC25A15 gene. ORNT1 is a transporter protein that transports ornithine across the inner mitochondrial membrane to the mitochondrial matrix, where it participates in the urea cycle. Failure to transport ornithine results in an interruption of the urea cycle and the accumulation of ammonia. Approximately 17 mutations in the SLC25A15 gene have been identified in individuals with ORNT1 deficiency, including F188delta, E180K, T32R, Q89X, G27R, G190D, R275Q, and a 13q14 microdeletion, etc (Camacho et al., Nat Genet. 1999 Jun;22(2):151-8; Camacho et al., Pediatric Research (2006) 60, 423-429; Salvi et al., Human Mutation, Mutation in Brief #457 (2001), which are hereby incorporated by reference in their entirety). Affected newborns typically present with lethargy, muscular hypotonia, and seizures. If untreated, death occurs within the first few days. The majority of survivors have pyramidal tract signs, with spastic paraparesis (Lemay et al., J Pediatr. 1992 Nov;121(5 Pt 1):725-30, Salvi et al., Neurology. 2001;57(5):911, which are hereby incorporated by reference in their entirety). Most have myoclonic seizures, ataxia, and mental retardation. A milder form of the disorder has been reported in adults, who become symptomatic following protein-rich meals.

[0065] In some embodiments, methods of the present invention involve modulating the expression of the SLC25A15 gene. SLC25A15 may also be referred to as Solute Carrier Family 25 Member 15, Solute Carrier Family 25 (Mitochondrial Carrier, Ornithine Transporter) Member 15, Ornithine Transporter 1, ORNT1, Mitochondrial Ornithine Transporter 1, D13S327, ORC1, and HHH. SLC25A15 has a cytogenetic location of 13q14.11 and the genomic coordinate are on Chromosome 13 on the forward strand at position 40,789,412-40,810,111. MRPS31 (ENSG00000102738) is the gene upstream of SLC25A15 and MIR621 (ENSG00000207652) is the genes downstream of SLC25A15. TPTE2P5 (ENSG00000168852) is a gene that overlaps with SLC25A15 on the reserve strand. SLC25A15 has a NCBI gene ID of 10166, Uniprot ID of

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Q9Y619 and Ensembl Gene ID of ENSG00000102743. The genomic sequence of SLC25A15 is shown as in SEQ ID NO: 7.

Citrin deficiency

[0066] In some embodiments, methods and compositions of the present invention may be used to treat Citrin deficiency. Citrin deficiency (neonatal-onset MIM #605814 and adult-onset #603471), also known as Citrullinemia type II, is an autosomal recessive disorder caused by mutations in the SLC25A13 gene. Citrin is a transporter protein responsible for the transport of aspartate into the urea cycle. The loss of citrin blocks the aspartate transport and decrease the ability of ASS to produce argininosuccinate. More than 20 mutations in the SLC25A13 gene have been identified in people with adult-onset type II citrullinemia. It can manifest in newborns as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and in adults as recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2). Citrin deficiency as a cause of neonatal intrahepatic cholestasis occurs almost exclusively in Asian infants (Yeh et al., J Pediatr 2006; 148:642; Zhang et al., Tohoku J Exp Med. 2014 Aug;233(4):275-81; Tzun and Marques., EC Paediatrics 2.5 (2016); Lin et al., Sci Rep. 2016 Jul 11;6:29732; which are hereby incorporated by reference in their entirety). Adult-onset form of this disease is characterized by fatty liver, hyperammonemia, and neurological symptoms (Yasuda et al., Hum Genet 2000; 107:537; Lee et al., J Pediatr Gastroenterol Nutr 2010; 50:682, which are hereby incorporated by reference in their entirety).

[0067] In some embodiments, methods of the present invention involve modulating the expression of the SLC25A13 gene. SLC25A13 may also be referred to as Solute Carrier Family 25 Member 13, Mitochondrial Aspartate Glutamate Carrier 2, Solute Carrier Family 25 (Aspartate/Glutamate Carrier) Member 13, ARALAR2, CITRIN, Calcium-Binding Mitochondrial Carrier Protein Aralar2, and CTLN2. SLC25A13 has a cytogenetic location of 7q21.3 and the genomic coordinate are on Chromosome 7 on the reverse strand at position 96,120,220-96,322,147. DYNC1H1 (ENSG00000158560) is the gene upstream of SLC25A13 and RNU6-532P (ENSG00000207045) is the gene downstream of SLC25A13. CYCSP18, MIR591 (ENSG00000208025), and RPL21P74 are genes located within SLC25A13. SLC25A13 has a NCBI gene ID of 10165, Uniprot ID of Q9UJS0 and Ensembl Gene ID of ENSG00000004864. The genomic sequence of SLC25A13 is shown as in SEQ ID NO: 8.

III. COMPOSITIONS AND METHODS OF THE INVENTION

[0068] The present invention provides compositions and methods for modulating the expression of one or more urea cycle-related genes to treat a urea cycle disorder. Any one or

more of the compositions and methods described herein may be used to treat a urea cycle disorder in a subject.

[0069] The terms “subject” and “patient” are used interchangeably herein and refer to an animal to whom treatment with the compositions according to the present invention is provided. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human being.

[0070] In some embodiments, subjects may have been diagnosed with or have symptoms for a urea cycle disorder, e.g., CPS1 deficiency, OTC deficiency, ASS1 Deficiency, ASL deficiency, NAGS deficiency, ARG1 deficiency, ORNT1 deficiency, and/or citrin deficiency. In other embodiments, subjects may be susceptible to or at risk for a urea cycle disorder, e.g., CPS1 deficiency, OTC deficiency, ASS1 Deficiency, ASL deficiency, NAGS deficiency, ARG1 deficiency, ORNT1 deficiency, and/or citrin deficiency.

[0071] In some embodiments, subjects may carry mutations within or near a urea cycle-related gene. In some embodiments, subjects may carry one or more mutations within or near the CPS1 gene. In some embodiments, subjects may carry one or more mutations within or near the OTC gene. In some embodiments, subjects may carry one or more mutations within or near the ASS1 gene. In some embodiments, subjects may carry one or more mutations within or near the ASL gene. In some embodiments, subjects may carry one or more mutations within or near the NAGS gene. In some embodiments, subjects may carry one or more mutations within or near the ARG1 gene. In some embodiments, subjects may carry one or more mutations within or near the SLC25A15 gene. In some embodiments, subjects may carry one or more mutations within or near the SLC25A13 gene. In some embodiment, subjects may carry one functional allele and one mutated allele of a urea cycle-related gene. In some embodiment, subjects may carry two mutated alleles of a urea cycle-related gene.

[0072] In some embodiments, subjects may have dysregulated expression of at least one urea cycle-related gene. In some embodiments, subjects may have a deficiency of at least one urea cycle-related protein. In some embodiments, subjects may have at least one urea cycle-related protein that is partially functional.

[0073] In some embodiments, compositions and methods of the present invention may be used to increase the expression of a urea cycle-related gene in a cell or a subject. Changes in gene expression may be assessed at the RNA level or protein level by various techniques known in the art and described herein, such as RNA-seq, qRT-PCR, Western Blot, or enzyme-linked immunosorbent assay (ELISA). Changes in gene expression may be determined by dividing the level of target gene expression in the treated cell or subject by the level of expression in an untreated or control cell or subject. In some embodiments, compositions and methods of the

present invention cause an increase in the expression of a urea cycle-related gene by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 250%, at least about 300%, at least about 400%, at least about 500%, from about 25% to about 50%, from about 40% to about 60%, from about 50% to about 70%, from about 60% to about 80%, from about 80% to about 100%, from about 100% to about 125%, from about 100 to about 150%, from about 150% to about 200%, from about 200% to about 300%, from about 300% to about 400%, from about 400% to about 500%, or more than 500%. In some embodiments, compositions and methods of the present invention cause a fold change in the expression of a urea cycle-related gene by about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 12 fold, about 15 fold, about 18 fold, about 20 fold, about 25 fold, or more than 30 fold.

[0074] In some embodiments, the increase in the expression of a urea cycle-related gene induced by compositions and methods of the present invention may be sufficient to prevent or alleviate one or more signs or symptoms of a urea cycle disorder.

Small molecules

[0075] In some embodiments, compounds used to modulate the expression of a urea cycle-related gene may include small molecules. As used herein, the term “small molecule” refers a low molecular weight drug, i.e. <5000 Daltons organic compound that may help regulate a biological process. In some embodiments, small molecule compounds described herein are applied to a genomic system to interfere with components (e.g., transcription factor, signaling proteins) of the gene signaling networks associated with one or more urea cycle-related genes, thereby modulating the expression of these genes. In some embodiments, small molecule compounds described herein are applied to a genomic system to alter the boundaries of an insulated neighborhood and/or disrupt signaling centers associated with one or more urea cycle-related genes, thereby modulating the expression of these genes.

[0076] A small molecule screen may be performed to identify small molecules that act through signaling centers of an insulated neighborhood to alter gene signaling networks which may modulate expression of a select group of urea cycle-related genes. For example, known signaling agonists/antagonists may be administered. Credible hits are identified and validated by the small molecules that are known to work through a signaling center and modulate expression of the target gene.

[0077] In some embodiments, small molecule compounds capable of modulating expression of one or more urea cycle-related genes include, but are not limited to, 17-AAG (Tanespimycin),

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Afatinib, Amlodipine Besylate, Amuvatinib, AZD2858, BAY 87-2243, BIRB 796, bms-986094 (inx-189), Bosutinib, Calcitriol, CD 2665, Ceritinib, CI-4AS-1, CO-1686 (Rociletinib), CP-673451, Crenolanib, Crizotinib, Darapladib, Dasatinib, Deoxycorticosterone, Echinomycin, Enzastaurin, Epinephrine, Erlotinib, EVP-6124 (hydrochloride) (encenicline), EW-7197, FRAX597, GDC-0879, GO6983, GSK2334470, GZD824 Dimesylate, INNO-206 (aldoxorubicin), LDN193189, LDN-212854, Merestinib, MK-0752, Momelotinib, Oligomycin A, OSU-03012, Pacritinib (SB1518), PHA-665752, Phenformin, Phorbol 12,13-dibutyrate, Pifithrin- μ , PND-1186, prednisone, R788 (fostamatinib disodium hexahydrate), Rifampicin, Semaxinib, SIS3, SKL2001, SMI-4a, T0901317, TFP, Thalidomide, Tivozanib, TP-434 (Eravacycline), WYE-125132 (WYE-132), and Zibotentan, or derivatives or analogs thereof.

Any one of these compounds or a combination thereof may be administered to a subject to treat a urea cycle disorder, such as CPS1 deficiency, OTC deficiency, ASS1 Deficiency, ASL deficiency, NAGS deficiency, ARG1 deficiency, ORNT1 deficiency, and/or citrin deficiency.

[0078] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include 17-AAG (Tanespimycin), or a derivative or an analog thereof. 17-AAG (Tanespimycin), also known as NSC 330507 or CP 127374, is a potent HSP90 inhibitor with half-maximal inhibitory concentration (IC_{50}) of 5 nM, a 100-fold higher binding affinity for HSP90 derived from tumor cells than HSP90 from normal cells.

[0079] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Afatinib, or a derivative or an analog thereof. Afatinib, also known as BIBW2992, irreversibly inhibits epidermal growth factor receptor (EGFR)/HER2 including EGFR (wildtype), EGFR (L858R), EGFR (L858R/T790M) and HER2 with IC_{50} of 0.5 nM, 0.4 nM, 10 nM and 14 nM, respectively. It is 100-fold more active against Gefitinib-resistant L858R-T790M EGFR mutant.

[0080] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Amlodipine Besylate, or a derivative or an analog thereof. Amlodipine, also known as Norvasc, is a long-acting calcium channel blocker with an IC_{50} of 1.9 nM.

[0081] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Amuvatinib, or a derivative or an analog thereof. Amuvatinib, also known as MP-470, is a potent and multi-targeted inhibitor of c-Kit, PDGFR α and FLT3 with IC_{50} of 10 nM, 40 nM and 81 nM, respectively.

[0082] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include AZD2858, or a derivative or an analog thereof.

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AZD2858 is a selective GSK-3 inhibitor with an IC₅₀ of 68 nM. It activates Wnt signaling and increases bone mass in rats.

[0083] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include BAY 87-2243, or a derivative or an analog thereof. BAY 87-2243 is a potent and selective hypoxia-inducible factor-1 (HIF-1) inhibitor.

[0084] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include BIRB 796, or a derivative or an analog thereof. BIRB 796, also known as Doramapimod, is a highly selective p38 α MAPK inhibitor with dissociation constant (K_d) of 0.1 nM, 330-fold greater selectivity versus JNK2. It shows weak inhibition for c-RAF, Fyn and Lck and insignificant inhibition of ERK-1, SYK, IKK2, ZAP-70, EGFR, HER2, PKA, PKC, and PKC $\alpha/\beta/\gamma$.

[0085] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include bms-986094 (inx-189), or a derivative or an analog thereof. Bms-986094, also known as INX-08189, INX-189, or IDX-189, is a prodrug of a guanosine nucleotide analogue (2'-C-methylguanosine). Bms-986094 is an RNA-directed RNA polymerase (NS5B) inhibitor originally developed by Inhibitex (acquired by Bristol-Myers Squibb in 2012). It was in phase II clinical trials for the treatment of hepatitis C virus infection. However, the study was discontinued due to unexpected cardiac and renal adverse events.

[0086] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Bosutinib, or a derivative or an analog thereof. Bosutinib, also known as SKI-606, is a novel, dual Src/Abl inhibitor with IC₅₀ of 1.2 nM and 1 nM, respectively.

[0087] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Calcitriol, or a derivative or an analog thereof. Calcitriol, also known as 1,25-Dihydroxyvitamin D₃ or Rocaltrol, is the hormonally active form of vitamin D. Calcitriol is the active metabolite of vitamin D₃ that activates the vitamin D receptor.

[0088] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include CD 2665, or a derivative or an analog thereof. CD 2665 is a selective RAR $\beta\gamma$ antagonist with K_d values of 110 nM, 306 nM, and > 1000 nM for RAR γ , RAR β , and RAR α , respectively. It blocks retinoic acid-induced apoptosis *ex vivo*.

[0089] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Ceritinib, or a derivative or an analog thereof. Ceritinib, also known as LDK378, is potent inhibitor against ALK with IC₅₀ of 0.2 nM, exhibiting 40- and 35-fold selectivity against IGF-1R and InsR, respectively.

[0090] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include CI-4AS-1, or a derivative or an analog thereof. CI-4AS-1 is a potent steroidal androgen receptor agonist ($IC_{50} = 12$ nM). It mimics the action of 5 α -dihydrotestosterone (DHT). It transactivates the mouse mammary tumor virus (MMTV) promoter; represses MMP1 promoter activity. It also inhibits 5 α -reductase type I and II with IC_{50} values of 6 nM and 10 nM, respectively.

[0091] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include CO-1686 (Rociletinib), or a derivative or an analog thereof. CO-1686, also known as Rociletinib, is a novel, irreversible and orally delivered kinase inhibitor that specifically targets the mutant forms of EGFR including T790M ($IC_{50}=21$ nM).

[0092] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include CP-673451, or a derivative or an analog thereof. CP 673451 is a selective inhibitor of PDGFR α/β with IC_{50} of 10 nM/1 nM, exhibiting >450-fold selectivity over other angiogenic receptors. CP 673451 also has antiangiogenic and antitumor activity.

[0093] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Crenolanib, or a derivative or an analog thereof. Crenolanib, also known as CP-868596, is a potent and selective inhibitor of PDGFR α/β with K_d of 2.1 nM/3.2 nM. It also potently inhibits FLT3 and is sensitive to D842V mutation not V561D mutation. It is >100-fold more selective for PDGFR than c-Kit, VEGFR-2, TIE-2, FGFR-2, EGFR, erbB2, and Src.

[0094] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Crizotinib, or a derivative or an analog thereof. Crizotinib, also known as PF-2341066, is a potent inhibitor of c-Met and ALK with IC_{50} of 11 nM and 24 nM, respectively.

[0095] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Darapladib, or a derivative or an analog thereof. Darapladib is a selective and orally active inhibitor of lipoprotein-associated phospholipase A2 (Lp-PLA2) with IC_{50} of 270 pM. Lp-PLA2 may link lipid metabolism with inflammation, leading to the increased stability of atherosclerotic plaques present in the major arteries. Darapladib is being studied as a possible add-on treatment for atherosclerosis.

[0096] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Dasatinib, or a derivative or an analog thereof. Dasatinib is a novel, potent and multi-targeted inhibitor that targets Abl, Src, and c-Kit, with IC_{50} of < 1 nM, 0.8 nM, and 79 nM, respectively.

[0097] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Deoxycorticosterone, or a derivative or an analog thereof. Deoxycorticosterone acetate is a steroid hormone used for intramuscular injection for replacement therapy of the adrenocortical steroid. 11 β -hydroxylation of deoxycorticosterone leads to corticosterone.

[0098] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Echinomycin, or a derivative or an analog thereof. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that controls genes involved in glycolysis, angiogenesis, migration, and invasion. Echinomycin is a cell-permeable inhibitor of HIF-1-mediated gene transcription. It acts by intercalating into DNA in a sequence-specific manner, blocking the binding of either HIF-1 α or HIF-1 β to the hypoxia-responsive element. Echinomycin reversibly inhibits hypoxia-induced HIF-1 transcription activity in U215 cells with a half maximal effective concentration (EC₅₀) value of 1.2 nM. It inhibits hypoxia-induced expression of vascular endothelial growth factor, blocking angiogenesis and altering excitatory synaptic transmission in hippocampal neurons. Echinomycin also impairs expression of survivin, enhancing the sensitivity of multiple myeloma cells to melphalan.

[0099] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Enzastaurin, or a derivative or an analog thereof. Enzastaurin, also known as LY317615, is a potent PKC β selective inhibitor with IC₅₀ of 6 nM, exhibiting 6- to 20-fold selectivity against PKC α , PKC γ and PKC ϵ .

[00100] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Epinephrine, or a derivative or an analog thereof. Epinephrine HCl is a hormone and a neurotransmitter.

[00101] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Erlotinib, or a derivative or an analog thereof. Erlotinib is an EGFR inhibitor with IC₅₀ of 2 nM, >1000-fold more sensitive for EGFR than human c-Src or v-Abl.

[00102] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include EVP-6124 (hydrochloride) (encenicline), or a derivative or an analog thereof. EVP-6124 hydrochloride, also known as encenicline, is a novel partial agonist of α 7 neuronal nicotinic acetylcholine receptors (nAChRs). EVP-6124 shows selectivity for α 7 nAChRs and does not activate or inhibit heteromeric α 4 β 2 nAChRs.

[00103] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include EW-7197. EW-7197 is a highly potent, selective, and

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orally bioavailable TGF- β receptor ALK4/ALK5 inhibitor with IC₅₀ of 13 nM and 11 nM, respectively.

[00104] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include FRAX597, or a derivative or an analog thereof. FRAX597 is a potent, ATP-competitive inhibitor of group I PAKs with IC₅₀ of 8 nM, 13 nM, and 19 nM for PAK1, PAK2, and PAK3, respectively.

[00105] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GDC-0879, or a derivative or an analog thereof. GDC-0879 is a novel, potent, and selective B-Raf inhibitor with IC₅₀ of 0.13 nM with activity against c-Raf as well.

[00106] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GO6983, or a derivative or an analog thereof. GO6983 is a pan-PKC inhibitor against for PKC α , PKC β , PKC γ and PKC δ with IC₅₀ of 7 nM, 7 nM, 6 nM and 10 nM, respectively. It is less potent to PKC ζ and inactive to PKC μ .

[00107] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GSK2334470, or a derivative or an analog thereof. GSK2334470 is a novel PDK1 inhibitor with IC₅₀ of about 10 nM and with no activity at other close related AGC-kinases.

[00108] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GZD824 Dimesylate, or a derivative or an analog thereof. GZD824 is a novel orally bioavailable Bcr-Abl inhibitor for Bcr-Abl (wildtype) and Bcr-Abl (T315I) with IC₅₀ of 0.34 nM and 0.68 nM, respectively.

[00109] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include INNO-206 (aldoxorubicin), or a derivative or an analog thereof. INNO-206, also known as Aldoxorubicin, is the 6-maleimidocaproyl hydrazone derivative prodrug of the anthracycline antibiotic doxorubicin (DOXO-EMCH) with antineoplastic activity.

[00110] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include LDN193189, or a derivative or an analog thereof. LDN193189 is a selective BMP signaling inhibitor that inhibits the transcriptional activity of the BMP type I receptors ALK2 and ALK3 with IC₅₀ of 5 nM and 30 nM, respectively, exhibiting 200-fold selectivity for BMP versus TGF-B.

[00111] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include LDN-212854, or a derivative or an analog thereof. LDN-212854 is a potent and selective BMP receptor inhibitor with IC₅₀ of 1.3 nM for ALK2,

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exhibiting about 2-, 66-, 1641-, and 7135-fold selectivity over ALK1, ALK3, ALK4, and ALK5, respectively.

[00112] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Merestinib, or a derivative or an analog thereof. Merestinib, also known as LY2801653, is a type-II ATP competitive, slow-off inhibitor of MET tyrosine kinase with a K_d of 2 nM, a pharmacodynamic residence time (K_{off}) of 0.00132 min^{-1} and half life ($t_{1/2}$) of 525 min.

[00113] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include MK-0752, or a derivative or an analog thereof. MK-0752 is a potent, reversible inhibitor of γ -secretase, reducing the cleavage of amyloid precursor protein (APP) to $A\beta_{40}$ in human neuroblastoma SH-SY5Y cells with an IC_{50} value of 5 nM. It is orally bioavailable and crosses the blood-brain barrier, as orally administered MK-0752 dose-dependently reduces the generation of new amyloid β protein in the brain of rhesus monkeys. Through its effects on the NOTCH pathway, MK-0752 reduces the number of breast cancer stem cells in tumor grafts, enhancing the efficacy of the chemotherapy drug docetaxel in mice with breast cancer tumors.

[00114] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Momelotinib, or a derivative or an analog thereof. Momelotinib, also known as CYT387, is an ATP-competitive inhibitor of JAK1/JAK2 with IC_{50} of 11 nM/18 nM and approximately 10-fold selectivity versus JAK3.

[00115] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Oligomycin A, or a derivative or an analog thereof. Oligomycin A is an inhibitor of ATP synthase, inhibits oxidative phosphorylation and all the ATP-dependent processes occurring on the coupling membrane of mitochondria.

[00116] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include OSU-03012, or a derivative or an analog thereof. OSU-03012 is a potent inhibitor of recombinant PDK-1 with IC_{50} of 5 μM and 2-fold increase in potency over OSU-02067.

[00117] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Pacritinib (SB1518), or a derivative or an analog thereof. Pacritinib, also known as SB1518, is a potent and selective inhibitor JAK2 and FLT3 with IC_{50} s of 23 and 22 nM in cell-free assays, respectively.

[00118] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include PHA-665752, or a derivative or an analog thereof.

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PHA-665752 is a potent, selective and ATP-competitive c-Met inhibitor with IC₅₀ of 9 nM, >50-fold selectivity for c-Met than RTKs or STKs.

[00119] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Phenformin, or a derivative or an analog thereof. Phenformin hydrochloride is a hydrochloride salt of phenformin that is an anti-diabetic drug from the biguanide class.

[00120] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Phorbol 12,13-dibutyrate, or a derivative or an analog thereof. Phorbol 12,13-dibutyrate is a protein kinase C activator. It induces contraction of vascular smooth muscle and inhibits MLC phosphatase (MLCP) in vascular smooth muscle. The activity does not alter intracellular Ca²⁺ concentration. It also inhibits the activity of Na⁺, K⁺ ATPase in opossum kidney cells.

[00121] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Pifithrin-μ, or a derivative or an analog thereof. Pifithrin-μ specifically inhibits p53 activity by reducing its affinity to Bcl-xL and Bcl-2, and it also inhibits HSP70 function and autophagy.

[00122] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include PND-1186, or a derivative or an analog thereof. PND-1186, VS-4718, is a reversible and selective focal adhesion kinase (FAK) inhibitor with IC₅₀ of 1.5 nM.

[00123] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include prednisone, or a derivative or an analog thereof. Prednisone is a synthetic glucocorticoid with anti-inflammatory and immunosuppressive activity.

[00124] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include R788 (fostamatinib disodium hexahydrate), or a derivative or an analog thereof. R788 sodium salt hydrate (fostamatinib), a prodrug of the active metabolite R406, is a potent Syk inhibitor with IC₅₀ of 41 nM.

[00125] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Rifampicin, or a derivative or an analog thereof. Rifampicin is a member of the rifamycin class of antibiotics, as it inhibits bacterial DNA-dependent RNA synthesis (K_i= ~1 nM). While this compound does not directly affect RNA synthesis in humans, its use as an antibiotic is limited by its potency toward activation of the pregnane X receptor (PXR, EC₅₀= ~2 μM), which results in the up-regulation of enzymes that alter drug metabolism. Access of rifampicin to the nuclear receptor PXR requires its import into the cell via organic anion transporters (OATs) in the OAT polypeptide (OATP) family. By acting

as a transporter substrate, rifampicin inhibits OATPs with K_i/IC_{50} values ranging from 0.58-18 μM .

[00126] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Semaxanib, or a derivative or an analog thereof. Semaxanib is a quinolone derivative with potential antineoplastic activity. Semaxanib reversibly inhibits ATP binding to the tyrosine kinase domain of vascular endothelial growth factor receptor 2 (VEGFR2).

[00127] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include SIS3, or a derivative or an analog thereof. SIS3 is a specific inhibitor of Smad3. It inhibits TGF- β and activin signaling by suppressing Smad3 phosphorylation without affecting the MAPK/p38, ERK, or PI3-kinase signaling pathways.

[00128] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include SKL2001, or a derivative or an analog thereof. SKL2001 is a novel agonist of the Wnt/ β -catenin pathway that disrupts the Axin/ β -catenin interaction.

[00129] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include SMI-4a, or a derivative or an analog thereof. SMI-4a is a potent inhibitor of Pim1 with IC_{50} of 17 nM, with modest potency to Pim-2. It does not significantly inhibit other serine/threonine- or tyrosine-kinases.

[00130] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include T0901317, or a derivative or an analog thereof. T0901317 is a potent, non-selective LXR agonist with EC_{50} of 50 nM. It increases ABCA1 expression associated with cholesterol efflux regulation and HDL metabolism. It also increases muscle expression of PPAR- δ and shows antiobesogenic effects *in vivo*.

[00131] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include TFP, or a derivative or an analog thereof. Trifluoperazine (TFP) has central antiadrenergic, antidopaminergic, and minimal anticholinergic effects. It is thought to function by blocking dopamine D1 and D2 receptors in the mesocortical and mesolimbic pathways, relieving or minimizing such symptoms of schizophrenia as hallucinations, delusions, and disorganized thought and speech.

[00132] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Thalidomide, or a derivative or an analog thereof. Thalidomide was introduced as a sedative drug, immunomodulatory agent and also is investigated for treating symptoms of many cancers. Thalidomide inhibits an E3 ubiquitin ligase, which is a CRBN-DDB1-Cul4A complex.

[00133] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Tivozanib, or a derivative or an analog thereof. Tivozanib, also known as AV-951, is a potent and selective VEGFR inhibitor for VEGFR1/2/3 with IC₅₀ of 30 nM/6.5 nM/15 nM. It also inhibits PDGFR and c-Kit but exhibits low activity against FGFR-1, Flt3, c-Met, EGFR and IGF-1R.

[00134] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include TP-434 (Eravacycline), or a derivative or an analog thereof. TP-434, also known as Eravacycline, is a novel, broad-spectrum fluorocycline antibiotic with activity against bacteria expressing major antibiotic resistance mechanisms including tetracycline-specific efflux and ribosomal-protection.

[00135] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include WYE-125132 (WYE-132), or a derivative or an analog thereof. WYE-125132, also known as WYE-132, is a highly potent, ATP-competitive mTOR inhibitor with IC₅₀ of 0.19 nM. It is highly selective for mTOR versus PI3Ks or PI3K-related kinases hSMG1 and ATR.

[00136] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Zibotentan, or a derivative or an analog thereof. Zibotentan, also known as ZD4054, is an orally administered, potent and specific endothelin A receptor (ETA)-receptor antagonist with IC₅₀ of 21 nM.

Polypeptides

[00137] In some embodiments, compounds for altering expression of a urea cycle-related gene comprise a polypeptide. As used herein, the term “polypeptide” refers to a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances, the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides and may be associated or linked. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analog of a corresponding naturally occurring amino acid.

[00138] In some embodiments, polypeptide compounds capable of modulating expression of one or more urea cycle-related genes include, but are not limited to, Activin, Anti mullerian

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hormone, BMP2, EGF, FGF, GDF10 (BMP3b), GDF2 (BMP9), HGF/SF, IGF-1, Nodal, PDGF, TNF- α , and Wnt3a, or derivatives or analogs thereof. Any one of these compounds or a combination thereof may be administered to a subject to treat a urea cycle disorder, such as CPS1 deficiency, OTC deficiency, ASS1 Deficiency, ASL deficiency, NAGS deficiency, ARG1 deficiency, ORNT1 deficiency, and/or citrin deficiency.

[00139] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Activin, or a derivative or an analog thereof. Activins are homodimers or heterodimers of the different β subunit isoforms, part of the transforming growth factor-beta (TGF-B) family. Mature Activin A has two 116 amino acids residues β A subunits (β A- β A). Activin displays an extensive variety of biological activities, including mesoderm induction, neural cell differentiation, bone remodeling, hematopoiesis, and reproductive physiology. Activins takes part in the production and regulation of hormones such as FSH, LH, GnRH and ACTH. Cells that are identified to express Activin A include fibroblasts, endothelial cells, hepatocytes, vascular smooth muscle cells, macrophages, keratinocytes, osteoclasts, bone marrow monocytes, prostatic epithelium, neurons, chondrocytes, osteoblasts, Leydig cells, Sertoli cells, and ovarian granulosa cells.

[00140] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include anti Mullerian hormone, or a derivative or an analog thereof. Anti Mullerian hormone is a member of the TGF-B gene family which mediates male sexual differentiation. Anti Mullerian hormone causes the regression of Mullerian ducts which would otherwise differentiate into the uterus and fallopian tubes. Some mutations in the anti-Mullerian hormone result in persistent Mullerian duct syndrome.

[00141] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include BMP2, or a derivative or an analog thereof. Bone morphogenetic protein 2 (BMP2) belongs to the TGF-B superfamily. The BMP family members are regulators of cell growth and differentiation in both embryonic and adult tissues. BMP2 is a candidate gene for the autosomal dominant disease of fibrodysplasia (myositis) ossificans progressiva.

[00142] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include EGF, or a derivative or an analog thereof. Epidermal Growth Factor (EGF) is a polypeptide growth factor which stimulates the proliferation of a wide range of epidermal and epithelial cells.

[00143] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include FGF, or a derivative or an analog thereof. Fibroblast Growth Factor- acidic (FGF-acidic), also known as FGF-1 and endothelial cell growth factor, is a

member of the FGF family which currently contain 23 members. FGF acidic and basic, unlike the other members of the family, lack signal peptides and are apparently secreted by mechanisms other than the classical protein secretion pathway. FGF acidic has been detected in large amounts in the brain. Other cells known to express FGF acidic include hepatocytes, vascular smooth muscle cells, CNS neurons, skeletal muscle cells, fibroblasts, keratinocytes, endothelial cells, intestinal columnar epithelium cells and pituitary basophils and acidophils.

[00144] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GDF10 (BMP3b), or a derivative or an analog thereof. GDF10, also known as BMP3b, is a member of the BMP family and the TGF-B superfamily. GDF10 is expressed in femur, brain, lung, skeletal, muscle, pancreas and testis, and has a role in head formation and possibly multiple roles in skeletal morphogenesis. In humans, GDF10 mRNA is found in the cochlea and lung of fetuses, and in testis, retina, pineal gland, and other neural tissues of adults. These proteins are characterized by a polybasic proteolytic processing site which is cleaved to produce a mature protein containing 7 conserved cysteine residues.

[00145] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include GDF2 (BMP9), or a derivative or an analog thereof. GDF2, also known as BMP9, is a member of BMP family and the TGF-B superfamily. BMP9 has a role in the maturation of basal forebrain cholinergic neurons (BFCN) as well as the induction and maintenance of the ability of these cells to respond to acetylcholine. BFCN are important for the processes of learning, memory and attention. BMP9 is a potent inducer of hepcidin (a cationic peptide that has an antimicrobial properties) in hepatocytes and can regulate iron metabolism. The physiological receptor of BMP9 is thought to be activin receptor-like kinase 1, ALK1 (also known as ACVRL1), an endothelial-specific type I receptor of the TGF-B receptor family. BMP9 is one of the most potent BMPs to induce orthotopic bone formation *in vivo*. BMP3, a blocker of most BMPs appears not to affect BMP9.

[00146] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include HGF/SF, or a derivative or an analog thereof. Hepatocyte Growth Factor (HGF), also known as hepatopoietin-A and scatter factor (SF), is a pleiotropic mitogen belonging to the peptidase S1 family (plasminogen subfamily). It is produced by mesenchymal cells and acts on epithelial cells, endothelial cells and hematopoietic progenitor cells. HGF binds to the proto-oncogenic c-Met receptor to activate a tyrosine kinase signaling cascade. It regulates cell growth, motility and morphogenesis, and it also plays a pivotal role in angiogenesis, tumorigenesis and tissue regeneration.

[00147] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include IGF-1, or a derivative or an analog thereof. Insulin-like growth factor I (IGF-I) also known as Somatomedin C is a hormone similar in molecular structure to insulin. Human IGF-I has two isoforms (IGF-IA and IGF-IB) which is differentially expressed by various tissues. Mature human IGF-I respectively shares 94% and 96% aa sequence identity with mouse and rat IGF-I. Both IGF-I and IGF-II (another ligand of IGF) can signal through the IGF-I receptor (IGFIR), but IGF-II can alone bind the IGF-II receptor (IGFIIR/Mannose-6-phosphate receptor). IGF-I plays an important role in childhood growth and continues to have anabolic effects in adults.

[00148] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Nodal, or a derivative or an analog thereof. Nodal is a 13 kDa member of the TGF- β superfamily of molecules. In human, it is synthesized as a 347 amino acid preproprecursor that contains a 26 amino acid signal sequence, a 211 amino acid prodomain, and a 110 amino acid mature region. Consistent with its TGF- β superfamily membership, it exists as a disulfide-linked homodimer and would be expected to demonstrate a cysteine-knot motif. Mature human Nodal is 99%, 98%, 96% and 98% amino acid identical to mature canine, rat, bovine and mouse Nodal, respectively. Nodal signals through two receptor complexes, both of which contain members of the TGF- β family of Ser/Thr kinase receptors.

[00149] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include PDGF, or a derivative or an analog thereof. the Platelet-derived growth factor (PDGF) is a disulfide-linked dimer consisting of two peptides-chain A and chain B. PDGF has three subforms: PDGF-AA, PDGF-BB, PDGF-AB. It is involved in a number of biological processes, including hyperplasia, embryonic neuron development, chemotaxis, and respiratory tubule epithelial cell development. The function of PDGF is mediated by two receptors (PDGFR α and PDGFR β).

[00150] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include TNF- α , or a derivative or an analog thereof. TNF- α , the prototypical member of the TNF protein superfamily, is a homotrimeric type-II membrane protein. Membrane bound TNF- α is cleaved by the metalloprotease TACE/ADAM17 to generate a soluble homotrimer. Both membrane and soluble forms of TNF- α are biologically active. TNF- α is produced by a variety of immune cells including T cells, B cells, NK cells and macrophages. Cellular response to TNF- α is mediated through interaction with receptors TNF-R1 and TNF-R2 and results in activation of pathways that favor both cell survival and apoptosis depending on the cell type and biological context. Activation of kinase pathways (including JNK, ERK (p44/42), p38 MAPK and NF- κ B) promotes the survival of cells, while TNF- α mediated activation of

caspase-8 leads to programmed cell death. TNF- α plays a key regulatory role in inflammation and host defense against bacterial infection, notably *Mycobacterium tuberculosis*. The role of TNF- α in autoimmunity is underscored by blocking TNF- α action to treat rheumatoid arthritis and Crohn's disease.

[00151] In some embodiments, compounds capable of modulating the expression of one or more urea cycle-related genes may include Wnt3a, or a derivative or an analog thereof. The WNT gene family consists of structurally related genes which encode secreted signaling proteins. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis. This gene is a member of the WNT gene family. It encodes a protein which shows 96% amino acid identity to mouse Wnt3a protein, and 84% to human WNT3 protein, another WNT gene product. This gene is clustered with WNT14 gene, another family member, in chromosome 1q42 region.

Antibodies

[00152] In some embodiments, compounds for altering expression of one or more urea cycle-related genes comprise an antibody. In one embodiment, antibodies of the present invention comprising antibodies, antibody fragments, their variants or derivatives described herein are specifically immunoreactive with at least one component of the gene signaling networks associated with the urea cycle-related gene.

[00153] As used herein, the term "antibody" is used in the broadest sense and specifically covers various embodiments including, but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies formed from at least two intact antibodies), and antibody fragments such as diabodies so long as they exhibit a desired biological activity. Antibodies are primarily amino-acid based molecules but may also comprise one or more modifications such as with sugar moieties.

[00154] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising an antigen binding region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site. Also produced is a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking antigen. Antibodies of the present invention may comprise one or more of these fragments. For the purposes herein, an "antibody" may comprise a heavy and light variable domain as well as an Fc region.

[00155] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[00156] As used herein, the term "variable domain" refers to specific antibody domains that differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. As used herein, the term "Fv" refers to antibody fragments which contain a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association.

[00157] Antibody "light chains" from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda based on amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

[00158] "Single-chain Fv" or "scFv" as used herein, refers to a fusion protein of V_H and V_L antibody domains, wherein these domains are linked together into a single polypeptide chain. In some embodiments, the Fv polypeptide linker enables the scFv to form the desired structure for antigen binding.

[00159] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain V_H connected to a light chain variable domain V_L in the same polypeptide chain. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993), the contents of each of which are incorporated herein by reference in their entirety.

[00160] Antibodies of the present invention may be polyclonal or monoclonal or recombinant, produced by methods known in the art or as described in this application. The term "monoclonal

antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous cells (or clones), i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

[00161] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. The monoclonal antibodies herein include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies.

[00162] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the hypervariable region from an antibody of the recipient are replaced by residues from the hypervariable region from an antibody of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity.

[00163] The term "hypervariable region" when used herein in reference to antibodies refers to regions within the antigen binding domain of an antibody comprising the amino acid residues that are responsible for antigen binding. The amino acids present within the hypervariable regions determine the structure of the complementarity determining region (CDR). As used herein, the "CDR" refers to the region of an antibody that comprises a structure that is complementary to its target antigen or epitope.

[00164] In some embodiments, the compositions of the present invention may be antibody mimetics. The term "antibody mimetic" refers to any molecule which mimics the function or effect of an antibody and which binds specifically and with high affinity to their molecular targets. As such, antibody mimics include nanobodies and the like.

[00165] In some embodiments, antibody mimetics may be those known in the art including, but are not limited to affibody molecules, affilins, affitins, anticalins, avimers, DARPins,

Fynomers and Kunitz and domain peptides. In other embodiments, antibody mimetics may include one or more non-peptide region.

[00166] As used herein, the term “antibody variant” refers to a biomolecule resembling an antibody in structure and/or function comprising some differences in their amino acid sequence, composition or structure as compared to a native antibody.

[00167] The preparation of antibodies, whether monoclonal or polyclonal, is known in the art. Techniques for the production of antibodies are well known in the art and described, e.g. in Harlow and Lane "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1988 and Harlow and Lane “Using Antibodies: A Laboratory Manual” Cold Spring Harbor Laboratory Press, 1999.

[00168] Antibodies of the present invention may be characterized by their target molecule(s), by the antigens used to generate them, by their function (whether as agonists or antagonists) and/or by the cell niche in which they function.

[00169] Measures of antibody function may be made relative to a standard under normal physiologic conditions, *in vitro* or *in vivo*. Measurements may also be made relative to the presence or absence of the antibodies. Such methods of measuring include standard measurement in tissue or fluids such as serum or blood such as Western blot, enzyme-linked immunosorbent assay (ELISA), activity assays, reporter assays, luciferase assays, polymerase chain reaction (PCR) arrays, gene arrays, Real Time reverse transcriptase (RT) PCR and the like.

[00170] Antibodies of the present invention exert their effects via binding (reversibly or irreversibly) to one or more target sites. While not wishing to be bound by theory, target sites which represent a binding site for an antibody, are most often formed by proteins or protein domains or regions. However, target sites may also include biomolecules such as sugars, lipids, nucleic acid molecules or any other form of binding epitope.

[00171] Alternatively, or additionally, antibodies of the present invention may function as ligand mimetics or nontraditional payload carriers, acting to deliver or ferry bound or conjugated drug payloads to specific target sites.

[00172] Changes elicited by antibodies of the present invention may result in a neomorphic change in the cell. As used herein, “a neomorphic change” is a change or alteration that is new or different. Such changes include extracellular, intracellular and cross cellular signaling.

[00173] In some embodiments, compounds or agents of the invention act to alter or control proteolytic events. Such events may be intracellular or extracellular.

[00174] Antibodies of the present invention, as well as antigens used to generate them, are primarily amino acid-based molecules. These molecules may be "peptides," "polypeptides," or "proteins.”

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[00175] As used herein, the term “peptide” refers to an amino-acid based molecule having from 2 to 50 or more amino acids. Special designators apply to the smaller peptides with “dipeptide” referring to a two amino acid molecule and “tripeptide” referring to a three amino acid molecule. Amino acid based molecules having more than 50 contiguous amino acids are considered polypeptides or proteins.

[00176] The terms "amino acid" and "amino acids" refer to all naturally occurring L-alpha-amino acids as well as non-naturally occurring amino acids. Amino acids are identified by either the one-letter or three-letter designations as follows: aspartic acid (Asp:D), isoleucine (Ile:I), threonine (Thr:T), leucine (Leu:L), serine (Ser:S), tyrosine (Tyr:Y), glutamic acid (Glu:E), phenylalanine (Phe:F), proline (Pro:P), histidine (His:H), glycine (Gly:G), lysine (Lys:K), alanine (Ala:A), arginine (Arg:R), cysteine (Cys:C), tryptophan (Trp:W), valine (Val:V), glutamine (Gln:Q) methionine (Met:M), asparagines (Asn:N), where the amino acid is listed first followed parenthetically by the three and one letter codes, respectively.

Hybridizing oligonucleotides

[00177] In some embodiments, oligonucleotides, including those which function via a hybridization mechanism, whether single or double stranded such as antisense molecules, RNAi constructs (including siRNA, saRNA, microRNA, etc.), aptamers and ribozymes may be used to alter or as perturbation stimuli of the gene signaling networks associated with a urea cycle-related gene.

[00178] As such oligonucleotides may also serve as therapeutics, their therapeutic liabilities and treatment outcomes may be ameliorated or predicted, respectively by interrogating the gene signaling networks of the invention.

Genome editing approaches

[00179] In certain embodiments, expression of a urea cycle-related gene may be modulated by altering the chromosomal regions defining the insulated neighborhood(s) and/or genome signaling center(s) associated with the urea cycle-related gene. For example, protein production may be increased by targeting a component of the gene signaling network that functions to repress the expression of the urea cycle-related gene.

[00180] Methods of altering the gene expression attendant to an insulated neighborhood include altering the signaling center (e.g. using CRISPR/Cas to change the signaling center binding site or repair/replace if mutated). These alterations may result in a variety of results including: activation of cell death pathways prematurely/inappropriately (key to many immune disorders), production of too little/much gene product (also known as the rheostat hypothesis), production of too little/much extracellular secretion of enzymes, prevention of lineage differentiation, switch of lineage pathways, promotion of stemness, initiation or interference with

auto regulatory feedback loops, initiation of errors in cell metabolism, inappropriate imprinting/gene silencing, and formation of flawed chromatin states. Additionally, genome editing approaches including those well-known in the art may be used to create new signaling centers by altering the cohesin necklace or moving genes and enhancers.

[00181] In certain embodiments, genome editing approaches describe herein may include methods of using site-specific nucleases to introduce single-strand or double-strand DNA breaks at particular locations within the genome. Such breaks can be and regularly are repaired by endogenous cellular processes, such as homology-directed repair (HDR) and non-homologous end joining (NHEJ). HDR is essentially an error-free mechanism that repairs double-strand DNA breaks in the presence of a homologous DNA sequence. The most common form of HDR is homologous recombination. It utilizes a homologous sequence as a template for inserting or replacing a specific DNA sequence at the break point. The template for the homologous DNA sequence can be an endogenous sequence (e.g., a sister chromatid), or an exogenous or supplied sequence (e.g., plasmid or an oligonucleotide). As such, HDR may be utilized to introduce precise alterations such as replacement or insertion at desired regions. In contrast, NHEJ is an error-prone repair mechanism that directly joins the DNA ends resulting from a double-strand break with the possibility of losing, adding or mutating a few nucleotides at the cleavage site. The resulting small deletions or insertions (termed “Indels”) or mutations may disrupt or enhance gene expression. Additionally, if there are two breaks on the same DNA, NHEJ can lead to the deletion or inversion of the intervening segment. Therefore, NHEJ may be utilized to introduce insertions, deletions or mutations at the cleavage site.

CRISPR/Cas systems

[00182] In certain embodiments, a CRISPR/Cas system may be used to delete CTCF anchor sites to modulate gene expression within the insulated neighborhood associated with that anchor site. See, Hnisz et al., Cell 167, November 17, 2016, which is hereby incorporated by reference in its entirety. Disruption of the boundaries of insulated neighborhood prevents the interactions necessary for proper function of the associated signaling centers. Changes in the expression genes that are immediately adjacent to the deleted neighborhood boundary have also been observed due to such disruptions.

[00183] In certain embodiments, a CRISPR/Cas system may be used to modify existing CTCF anchor sites. For example, existing CTCF anchor sites may be mutated or inverted by inducing NHEJ with a CRISPR/Cas nuclease and one or more guide RNAs, or masked by targeted binding with a catalytically inactive CRISPR/Cas enzyme and one or more guide RNAs. Alteration of existing CTCF anchor sites may disrupt the formation of existing insulated neighborhoods and alter the expression of genes located within these insulated neighborhoods.

[00184] In certain embodiments, a CRISPR/Cas system may be used to introduce new CTCF anchor sites. CTCF anchor sites may be introduced by inducing HDR at a selected site with a CRISPR/Cas nuclease, one or more guide RNAs and a donor template containing the sequence of a CTCF anchor site. Introduction of new CTCF anchor sites may create new insulated neighborhoods and/or alter existing insulated neighborhoods, which may affect expression of genes that are located adjacent to these insulated neighborhoods.

[00185] In certain embodiments, a CRISPR/Cas system may be used to alter signaling centers by changing signaling center binding sites. For example, if a signaling center binding site contains a mutation that affects the assembly of the signaling center with associated transcription factors, the mutated site may be repaired by inducing a double strand DNA break at or near the mutation using a CRISPR/Cas nuclease and one or more guide RNAs in the presence of a supplied corrected donor template.

[00186] In certain embodiments, a CRISPR/Cas system may be used to modulate expression of neighborhood genes by binding to a region within an insulated neighborhood (e.g., enhancer) and block transcription. Such binding may prevent recruitment of transcription factors to signaling centers and initiation of transcription. The CRISPR/Cas system may be a catalytically inactive CRISPR/Cas system that do not cleave DNA.

[00187] In certain embodiments, a CRISPR/Cas system may be used to knockdown expression of neighborhood genes via introduction of short deletions in coding regions of these genes. When repaired, such deletions would result in frame shifts and/or introduce premature stop codons in mRNA produced by the genes followed by the mRNA degradation via nonsense-mediated decay. This may be useful for modulation of expression of activating and repressive components of signaling pathways that would result in decreased or increased expression of genes under control of these pathways including disease genes such as CPS1, OTC, ASS, ASL, NAGS, and ARG1.

[00188] In other embodiments, a CRISPR/Cas system may also be used to alter cohesion necklace or moving genes and enhancers.

CRISPR/Cas enzymes

[00189] CRISPR/Cas systems are bacterial adaptive immune systems that utilize RNA-guided endonucleases to target specific sequences and degrade target nucleic acids. They have been adapted for use in various applications in the field of genome editing and/or transcription modulation. Any of the enzymes or orthologs known in the art or disclosed herein may be utilized in the methods herein for genome editing.

[00190] In certain embodiments, the CRISPR/Cas system may be a Type II CRISPR/Cas9 system. Cas9 is an endonuclease that functions together with a trans-activating CRISPR RNA (tracrRNA) and a CRISPR RNA (crRNA) to cleave double stranded DNAs. The two RNAs can

be engineered to form a single-molecule guide RNA by connecting the 3' end of the crRNA to the 5' end of tracrRNA with a linker loop. Jinek et al., *Science*, 337(6096):816-821 (2012) showed that the CRISPR/Cas9 system is useful for RNA-programmable genome editing, and international patent application WO2013/176772 provides numerous examples and applications of the CRISPR/Cas endonuclease system for site-specific gene editing, which are incorporated herein by reference in their entirety. Exemplary CRISPR/Cas9 systems include those derived from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Neisseria meningitidis*, *Treponema denticola*, *Streptococcus aureus*, and *Francisella tularensis*.

[00191] In certain embodiments, the CRISPR/Cas system may be a Type V CRISPR/Cpf1 system. Cpf1 is a single RNA-guided endonuclease that, in contrast to Type II systems, lacks tracrRNA. Cpf1 produces staggered DNA double-stranded break with a 4 or 5 nucleotide 5' overhang. Zetsche et al. *Cell*. 2015 Oct 22;163(3):759-71 provides examples of Cpf1 endonuclease that can be used in genome editing applications, which is incorporated herein by reference in its entirety. Exemplary CRISPR/Cpf1 systems include those derived from *Francisella tularensis*, *Acidaminococcus sp.*, and *Lachnospiraceae* bacterium.

[00192] In certain embodiments, nickase variants of the CRISPR/Cas endonucleases that have one or the other nuclease domain inactivated may be used to increase the specificity of CRISPR-mediated genome editing. Nickases have been shown to promote HDR versus NHEJ. HDR can be directed from individual Cas nickases or using pairs of nickases that flank the target area.

[00193] In certain embodiments, catalytically inactive CRISPR/Cas systems may be used to bind to target regions (e.g., CTCF anchor sites or enhancers) and interfere with their function. Cas nucleases such as Cas9 and Cpf1 encompass two nuclease domains. Mutating critical residues at the catalytic sites creates variants that only bind to target sites but do not result in cleavage. Binding to chromosomal regions (e.g., CTCF anchor sites or enhancers) may disrupt proper formation of insulated neighborhoods or signaling centers and therefore lead to altered expression of genes located adjacent to the target region.

[00194] In certain embodiments, a CRISPR/Cas system may include additional functional domain(s) fused to the CRISPR/Cas enzyme. The functional domains may be involved in processes including but not limited to transcription activation, transcription repression, DNA methylation, histone modification, and/or chromatin remodeling. Such functional domains include but are not limited to a transcriptional activation domain (e.g., VP64 or KRAB, SID or SID4X), a transcriptional repressor, a recombinase, a transposase, a histone remodeler, a DNA methyltransferase, a cryptochrome, a light inducible/controllable domain or a chemically inducible/controllable domain.

[00195] In certain embodiments, a CRISPR/Cas enzyme may be administered to a cell or a patient as one or a combination of the following: one or more polypeptides, one or more mRNAs encoding the polypeptide, or one or more DNAs encoding the polypeptide.

Guide nucleic acid

[00196] In certain embodiments, guide nucleic acids may be used to direct the activities of an associated CRISPR/Cas enzymes to a specific target sequence within a target nucleic acid. Guide nucleic acids provide target specificity to the guide nucleic acid and CRISPR/Cas complexes by virtue of their association with the CRISPR/Cas enzymes, and the guide nucleic acids thus can direct the activity of the CRISPR/Cas enzymes.

[00197] In one aspect, guide nucleic acids may be RNA molecules. In one aspect, guide RNAs may be single-molecule guide RNAs. In one aspect, guide RNAs may be chemically modified.

[00198] In certain embodiments, more than one guide RNAs may be provided to mediate multiple CRISPR/Cas-mediated activities at different sites within the genome.

[00199] In certain embodiments, guide RNAs may be administered to a cell or a patient as one or more RNA molecules or one or more DNAs encoding the RNA sequences.

Ribonucleoprotein complexes (RNPs)

[00200] In one embodiment, the CRISPR/Cas enzyme and guide nucleic acid may each be administered separately to a cell or a patient.

[00201] In another embodiment, the CRISPR/Cas enzyme may be pre-complexed with one or more guide nucleic acids. The pre-complexed material may then be administered to a cell or a patient. Such pre-complexed material is known as a ribonucleoprotein particle (RNP).

Zinc Finger Nucleases

[00202] In certain embodiments, genome editing approaches of the present invention involve the use of Zinc finger nucleases (ZFNs). Zinc finger nucleases (ZFNs) are modular proteins comprised of an engineered zinc finger DNA binding domain linked to a DNA-cleavage domain. A typical DNA-cleavage domain is the catalytic domain of the type II endonuclease FokI. Because FokI functions only as a dimer, a pair of ZFNs must be engineered to bind to cognate target “half-site” sequences on opposite DNA strands and with precise spacing between them to allow the two enable the catalytically active FokI domains to dimerize. Upon dimerization of the FokI domain, which itself has no sequence specificity per se, a DNA double-strand break is generated between the ZFN half-sites as the initiating step in genome editing.

Transcription Activator-Like Effector Nucleases (TALENs)

[00203] In certain embodiments, genome editing approaches of the present invention involve the use of Transcription Activator-Like Effector Nucleases (TALENs). TALENs represent another format of modular nucleases which, similarly to ZFNs, are generated by fusing an

engineered DNA binding domain to a nuclease domain, and operate in tandem to achieve targeted DNA cleavage. While the DNA binding domain in ZFN consists of Zinc finger motifs, the TALEN DNA binding domain is derived from transcription activator-like effector (TALE) proteins, which were originally described in the plant bacterial pathogen *Xanthomonas* sp. TALEs are comprised of tandem arrays of 33-35 amino acid repeats, with each repeat recognizing a single basepair in the target DNA sequence that is typically up to 20 bp in length, giving a total target sequence length of up to 40 bp. Nucleotide specificity of each repeat is determined by the repeat variable diresidue (RVD), which includes just two amino acids at positions 12 and 13. The bases guanine, adenine, cytosine and thymine are predominantly recognized by the four RVDs: Asn-Asn, Asn-Ile, His-Asp and Asn-Gly, respectively. This constitutes a much simpler recognition code than for zinc fingers, and thus represents an advantage over the latter for nuclease design. Nevertheless, as with ZFNs, the protein-DNA interactions of TALENs are not absolute in their specificity, and TALENs have also benefitted from the use of obligate heterodimer variants of the FokI domain to reduce off-target activity.

Modulation of urea cycle-related genes

[00204] Compositions and methods described herein may be effective in modulating the expression of one or more urea cycle-related genes. Such compositions and methods may be used to treat a urea cycle disorder.

[00205] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of CPS1. Compounds that may be used to modulate the CPS1 expression include, but are not limited to, Dasatinib, R788 (fostamatinib disodium hexahydrate), Bosutinib, Epinephrine, FRAX597, Merestinib, Deoxycorticosterone, 17-AAG (Tanespimycin), GDF2 (BMP9), GZD824 Dimesylate, PND-1186, Wnt3a, Nodal, Anti mullerian hormone, TNF- α , Activin, IGF-1, prednisone, PDGF, HGF/SF, EGF, BAY 87-2243, CP-673451, FGF, GDF10 (BMP3b), LDN193189, Amuvatinib, Momelotinib, Echinomycin, Pacritinib (SB1518), BMP2, Crizotinib, LDN-212854, Thalidomide, CO-1686 (Rociletinib), Zibotentan, and derivatives or analogs thereof. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate CPS1 expression. In some embodiments, R788 (fostamatinib disodium hexahydrate) perturbs the Protein Tyrosine Kinase/RTK signaling pathway to modulate CPS1 expression. In some embodiments, Bosutinib perturbs the Src signaling pathway to modulate CPS1 expression. In some embodiments, Epinephrine perturbs the Adrenergic receptor signaling pathway to modulate CPS1 expression. In some embodiments, FRAX597 perturbs the PAK signaling pathway to modulate CPS1 expression. In some embodiments, Merestinib perturbs the c-MET signaling pathway to modulate CPS1 expression. In some embodiments, Corticosterone perturbs the Mineralcorticoid

receptor signaling pathway to modulate CPS1 expression. In some embodiments, 17-AAG (Tanespimycin) perturbs the Cell Cycle/DNA Damage Metabolic Enzyme/Protease signaling pathway to modulate CPS1 expression. In some embodiments, GDF2 (BMP9) perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, GZD824 Dimesylate perturbs the ABL signaling pathway to modulate CPS1 expression. In some embodiments, PND-1186 perturbs the FAK signaling pathway to modulate CPS1 expression. In some embodiments, Wnt3a perturbs the WNT signaling pathway to modulate CPS1 expression. In some embodiments, Nodal perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, Anti mullerian hormone perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, TNF- α perturbs the NF-kB, MAPK, or Apoptosis pathway to modulate CPS1 expression. In some embodiments, Activin perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, IGF-1 perturbs the IGF-1R/InsR signaling pathway to modulate CPS1 expression. In some embodiments, prednisone perturbs the GR signaling pathway to modulate CPS1 expression. In some embodiments, PDGF perturbs the PDGFR signaling pathway to modulate CPS1 expression. In some embodiments, HGF/SF perturbs the c-MET signaling pathway to modulate CPS1 expression. In some embodiments, EGF perturbs the EGFR signaling pathway to modulate CPS1 expression. In some embodiments, BAY 87-2243 perturbs the Hypoxia activated signaling pathway to modulate CPS1 expression. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate CPS1 expression. In some embodiments, FGF perturbs the FGFR signaling pathway to modulate CPS1 expression. In some embodiments, GDF10 (BMP3b) perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, LDN193189 perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, Amuvatinib perturbs the PDGFR signaling pathway to modulate CPS1 expression. In some embodiments, Momelotinib perturbs the JAK/STAT signaling pathway to modulate CPS1 expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate CPS1 expression. In some embodiments, Pacritinib (SB1518) perturbs the JAK/STAT signaling pathway to modulate CPS1 expression. In some embodiments, BMP2 perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, Crizotinib perturbs the c-MET signaling pathway to modulate CPS1 expression. In some embodiments, LDN-212854 perturbs the TGF-B signaling pathway to modulate CPS1 expression. In some embodiments, Thalidomide perturbs the NF-kB signaling pathway to modulate CPS1 expression. In some embodiments, CO-1686 (Rociletinib) perturbs the JAK/STAT and/or Tyrosine Kinase/RTK signaling pathway to modulate CPS1 expression. In some embodiments, Zibotentan perturbs the GPCR/G protein signaling pathway to modulate CPS1 expression.

[00206] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the CPS1 gene. The CPS1 gene has a cytogenetic location of 2q34 and the genomic coordinate are on Chromosome 2 on the forward strand at position 210,477,682-210,679,107. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of CPS1. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, p300, and SMC1. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ONECUT2, and YY1. The signaling proteins may include but are not limited to TCF7L2, ESRA, FOS, NR3C1, JUN, NR5A2, RBPJK, RXR, STAT3, NR1H1, NF- κ B, SMAD2/3, SMAD4, STAT1, TEAD1, and TP53.

[0010] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of OTC. Compounds that may be used to modulate the OTC expression include, but are not limited to, CP-673451, Pacritinib (SB1518), Echinomycin, Crenolanib, Thalidomide, Amuvatinib, Dasatinib, Momelotinib, Activin, Wnt3a, INNO-206 (aldoxorubicin), TNF- α , Anti mullerian hormone, Pifithrin- μ , PDGF, IGF-1, FRAX597, Nodal, EGF, FGF, HGF/SF, BIRB 796, and derivatives or analogs thereof. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate OTC expression. In some embodiments, Pacritinib (SB1518) perturbs the JAK/STAT signaling pathway to modulate OTC expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate OTC expression. In some embodiments, Crenolanib perturbs the PDGFR signaling pathway to modulate OTC expression. In some embodiments, Thalidomide perturbs the NF- κ B signaling pathway to modulate OTC expression. In some embodiments, Amuvatinib perturbs the PDGFR signaling pathway to modulate OTC expression. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate OTC expression. In some embodiments, Momelotinib perturbs the JAK/STAT signaling pathway to modulate OTC expression. In some embodiments, Activin perturbs the TGF-B signaling pathway to modulate OTC expression. In some embodiments, Wnt3a perturbs the WNT signaling pathway to modulate OTC expression. In some embodiments, INNO-206 (aldoxorubicin) perturbs the Cell Cycle/DNA Damage pathway to modulate OTC expression. In some embodiments, TNF- α perturbs the NF- κ B, MAPK, or Apoptosis signaling pathway to modulate OTC expression. In some embodiments, Anti mullerian hormone perturbs the TGF-B signaling pathway to modulate OTC expression. In some embodiments, Pifithrin- μ perturbs the p53 signaling pathway to

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modulate OTC expression. In some embodiments, PDGF perturbs the PDGFR signaling pathway to modulate OTC expression. In some embodiments, IGF-1 perturbs the IGF-1R/InsR signaling pathway to modulate OTC expression. In some embodiments, FRAX597 perturbs the PAK signaling pathway to modulate OTC expression. In some embodiments, Nodal perturbs the TGF- β signaling pathway to modulate OTC expression. In some embodiments, EGF perturbs the EGFR signaling pathway to modulate OTC expression. In some embodiments, FGF perturbs the FGFR signaling pathway to modulate OTC expression. In some embodiments, HGF/SF perturbs the c-MET signaling pathway to modulate OTC expression. In some embodiments, BIRB 796 perturbs the MAPK signaling pathway to modulate OTC expression.

[00207] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the OTC gene. The OTC gene has a cytogenetic location of Xp11.4 and the genomic coordinate are on Chromosome X on the forward strand at position 38,352,545-38,421,450. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of OTC. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac and BRD4. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, and HNF1A. The signaling proteins may include but are not limited to TCF7L2, HIF1 α , ESRA, NR3C1, JUN, RXR, STAT3, NF- κ B, SMAD2/3, SMAD4, and TEAD1.

[00208] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of ASS1. Compounds that may be used to modulate the ASS1 expression include, but are not limited to, Dasatinib, CP-673451, Echinomycin, GDF2 (BMP9), Pacritinib (SB1518), Epinephrine, FRAX597, Bosutinib, TP-434 (Eravacycline), BMP2, SMI-4a, Amuvatinib, Crenolanib, Deoxycorticosterone, INNO-206 (aldoxorubicin), TNF- α , T0901317, and derivatives or analogs thereof. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate ASS1 expression. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate ASS1 expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate ASS1 expression. In some embodiments, GDF2 (BMP9) perturbs the TGF- β signaling pathway to modulate ASS1 expression. In some embodiments, Pacritinib (SB1518) perturbs the JAK/STAT signaling pathway to modulate ASS1 expression. In some embodiments, Epinephrine perturbs the Adrenergic receptor signaling pathway to modulate ASS1 expression. In some embodiments, FRAX597 perturbs the PAK signaling pathway to modulate ASS1 expression. In

some embodiments, Bosutinib perturbs the Src signaling pathway to modulate ASS1 expression. In some embodiments, TP-434 (Eravacycline) perturbs the Tetracycline-specific efflux signaling pathway to modulate ASS1 expression. In some embodiments, BMP2 perturbs the TGF- β signaling pathway to modulate ASS1 expression. In some embodiments, SMI-4a perturbs the PIM signaling pathway to modulate ASS1 expression. In some embodiments, Amuvatinib perturbs the PDGFR signaling pathway to modulate ASS1 expression. In some embodiments, Crenolanib perturbs the PDGFR signaling pathway to modulate ASS1 expression. In some embodiments, Corticosterone perturbs the Mineralcorticoid receptor signaling pathway to modulate ASS1 expression. In some embodiments, INNO-206 (aldoxorubicin) perturbs the Cell Cycle/DNA Damage pathway to modulate ASS1 expression. In some embodiments, TNF- α perturbs the NF- κ B, MAPK, or apoptosis pathway to modulate ASS1 expression. In some embodiments, T0901317 perturbs the LXR signaling pathway to modulate ASS1 expression.

[00209] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the ASS1 gene. The ASS1 gene has a cytogenetic location of 9q34.11 and the genomic coordinate are on Chromosome 9 on the forward strand at position 130,444,929-130,501,274. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of ASS1. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, p300, and SMC1. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, MYC, and YY1. The signaling proteins may include but are not limited to CREB1, NR1H4, HIF1a, ESRA, JUN, RXR, STAT3, NR1H1, NF- κ B, NR3C1, SMAD2/3, SMAD4, and TEAD1.

[00210] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of ASL. Compounds that may be used to modulate the ASL expression include, but are not limited to, CP-673451, Echinomycin, Pacritinib (SB1518), Dasatinib, Oligomycin A, Merestinib, Amuvatinib, Crenolanib, Epinephrine, BAY 87-2243, Thalidomide, and derivatives or analogs thereof. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate ASL expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate ASL expression. In some embodiments, Pacritinib (SB1518) perturbs the JAK/STAT signaling pathway to modulate ASL expression. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate ASL expression. In some embodiments, Oligomycin A perturbs the ATP channel signaling pathway to modulate ASL expression. In some embodiments,

Merestinib perturbs the c-MET signaling pathway to modulate ASL expression. In some embodiments, Amuvatinib perturbs the PDGFR signaling pathway to modulate ASL expression. In some embodiments, Crenolanib perturbs the PDGFR signaling pathway to modulate ASL expression. In some embodiments, Epinephrine perturbs the Adrenergic receptor signaling pathway to modulate ASL expression. In some embodiments, BAY 87-2243 perturbs the Hypoxia activated signaling pathway to modulate ASL expression. In some embodiments, Thalidomide perturbs the NF-kB signaling pathway to modulate ASL expression.

[00211] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the ASL gene. The ASL gene has a cytogenetic location of 7q11.21 and the genomic coordinate are on Chromosome 7 on the forward strand at position 66,075,798-66,093,558. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of ASL. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, and p300. The transcription factors may include but are not limited to HNF3, HNF4A, ONECUT1, HNF1A, and MYC. The signaling proteins may include but are not limited to TCF7L2, CREB1, NR1H4, HIF1a, ESRA, FOS, JUN, RBPJK, RXR, STAT3, NR1H1, NF-kB, NR3C1, SMAD2/3, SMAD4, STAT1, TEAD1, and TP53.

[0011] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of NAGS. Compounds that may be used to modulate the NAGS expression include, but are not limited to, AZD2858, Enzastaurin, Bosutinib, Semaxanib, INNO-206 (aldoxorubicin), TP-434 (Eravacycline), Phenformin, Crizotinib, SMI-4a, Dasatinib, Calcitriol, Pifithrin- μ , PHA-665752, Darapladib, Thalidomide, CO-1686 (Rociletinib), OSU-03012, prednisone, GSK2334470, Afatinib, Tivozanib, SKL2001, GDC-0879, EVP-6124 (hydrochloride) (encenicline), Amlodipine Besylate, T0901317, GO6983, Activin, WYE-125132 (WYE-132), SIS3, GDF2 (BMP9), Phorbol 12,13-dibutyrate, CD 2665, Erlotinib, Ceritinib, BMP2, TFP, HGF/SF, CI-4AS-1, and derivatives or analogs thereof. In some embodiments, AZD2858 perturbs the GSK-3 signaling pathway to modulate NAGS expression. In some embodiments, Enzastaurin perturbs the Epigenetics or TGF-beta/Smad signaling pathway to modulate NAGS expression. In some embodiments, Bosutinib perturbs the Src signaling pathway to modulate NAGS expression. In some embodiments, Semaxanib perturbs the VEGFR signaling pathway to modulate NAGS expression. In some embodiments, INNO-206 (aldoxorubicin) perturbs the Cell Cycle/DNA Damage pathway to modulate NAGS expression.

In some embodiments, TP-434 (Eravacycline) perturbs the Tetracycline-specific efflux signaling pathway to modulate NAGS expression. In some embodiments, Phenformin perturbs the AMPK signaling pathway to modulate NAGS expression. In some embodiments, Crizotinib perturbs the c-MET signaling pathway to modulate NAGS expression. In some embodiments, SMI-4a perturbs the PIM signaling pathway to modulate NAGS expression. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate NAGS expression. In some embodiments, Calcitriol perturbs the Vitamin D Receptor signaling pathway to modulate NAGS expression. In some embodiments, Pifithrin- μ perturbs the p53 signaling pathway to modulate NAGS expression. In some embodiments, PHA-665752 perturbs the c-MET signaling pathway to modulate NAGS expression. In some embodiments, Thalidomide perturbs the NF- κ B signaling pathway to modulate NAGS expression. In some embodiments, CO-1686 (Rociletinib) perturbs the JAK/STAT or Tyrosine Kinase/RTK signaling pathway to modulate NAGS expression. In some embodiments, OSU-03012 perturbs the PDK-1 signaling pathway to modulate NAGS expression. In some embodiments, prednisone perturbs the GR signaling pathway to modulate NAGS expression. In some embodiments, GSK2334470 perturbs the PDK-1 signaling pathway to modulate NAGS expression. In some embodiments, Afatinib perturbs the EGFR signaling pathway to modulate NAGS expression. In some embodiments, Tivozanib perturbs the Protein Tyrosine Kinase/RTK signaling pathway to modulate NAGS expression. In some embodiments, SKL2001 perturbs the WNT signaling pathway to modulate NAGS expression. In some embodiments, GDC-0879 perturbs the MAPK signaling pathway to modulate NAGS expression. In some embodiments, EVP-6124 (hydrochloride) (encenicline) perturbs the Membrane Transporter/Ion Channel signaling pathway to modulate NAGS expression. In some embodiments, Amlodipine Besylate perturbs the Calcium channel signaling pathway to modulate NAGS expression. In some embodiments, T0901317 perturbs the LXR signaling pathway to modulate NAGS expression. In some embodiments, GO6983 perturbs the PKC signaling pathway to modulate NAGS expression. In some embodiments, Activin perturbs the TGF- β signaling pathway to modulate NAGS expression. In some embodiments, WYE-125132 (WYE-132) perturbs the mTOR signaling pathway to modulate NAGS expression. In some embodiments, SIS3 perturbs the TGF- β signaling pathway to modulate NAGS expression. In some embodiments, GDF2 (BMP9) perturbs the TGF- β signaling pathway to modulate NAGS expression. In some embodiments, Phorbol 12,13-dibutyrate perturbs the PKC signaling pathway to modulate NAGS expression. In some embodiments, CD 2665 perturbs the RAR signaling pathway to modulate NAGS expression. In some embodiments, Erlotinib perturbs the EGFR signaling pathway to modulate NAGS expression. In some embodiments, Ceritinib perturbs the ALK signaling pathway to modulate NAGS expression. In some embodiments, BMP2 perturbs

the TGF- β signaling pathway to modulate NAGS expression. In some embodiments, TFP perturbs the Calmodulin signaling pathway to modulate NAGS expression. In some embodiments, HGF/SF perturbs the c-MET signaling pathway to modulate NAGS expression. In some embodiments, CI-4AS-1 perturbs the Androgen receptor signaling pathway to modulate NAGS expression.

[00212] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the NAGS gene. The NAGS gene has a cytogenetic location of 17q21.31 and the genomic coordinate are on Chromosome 17 on the forward strand at position 44,004,546-44,009,063. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of NAGS. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, and p300. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, and HNF1A. The signaling proteins may include but are not limited to TCF7L2, HIF1a, AHR, ESRA, JUN, RXR, STAT3, NR1H1, NF- κ B, NR3C1, SMAD2/3, SMAD4, TEAD1, and TP53.

[0012] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of ARG1. Compounds that may be used to modulate the ARG1 expression include, but are not limited to, R788 (fostamatinib disodium hexahydrate), Dasatinib, CP-673451, Merestinib, Echinomycin, Amuvatinib, Epinephrine, Bosutinib, Wnt3a, Anti mullerian Hormone, Nodal, Activin, IGF-1, 17-AAG (Tanespimycin), TNF- α , Pifithrin- μ , PDGF, Pacritinib (SB1518), GDF2 (BMP9), Crenolanib, prednisone, HGF/SF, Mometinib, EGF, Deoxycorticosterone, FGF, Thalidomide, Phenformin, Tivozanib, BAY 87-2243, GZD824 Dimesylate, GDF10 (BMP3b), PND-1186, FRAX597, BMP2, Oligomycin A, Rifampicin, MK-0752, and derivatives or analogs thereof. In some embodiments, R788 (fostamatinib disodium hexahydrate) perturbs the Protein Tyrosine Kinase/RTK signaling pathway to modulate ARG1 expression. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate ARG1 expression. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate ARG1 expression. In some embodiments, Merestinib perturbs the c-MET signaling pathway to modulate ARG1 expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate ARG1 expression. In some embodiments, Amuvatinib perturbs the PDGFR signaling pathway to modulate ARG1 expression. In some embodiments, Epinephrine perturbs the Adrenergic receptor signaling pathway to modulate ARG1 expression. In some embodiments,

Bosutinib perturbs the Src signaling pathway to modulate ARG1 expression. In some embodiments, Wnt3a perturbs the WNT signaling pathway to modulate ARG1 expression. In some embodiments, Anti mullerian Hormone perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, Nodal perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, Activin perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, IGF-1 perturbs the IGF-1R/InsR signaling pathway to modulate ARG1 expression. In some embodiments, 17-AAG (Tanespimycin) perturbs the Cell Cycle/DNA Damage Metabolic Enzyme/Protease signaling pathway to modulate ARG1 expression. In some embodiments, TNF-a perturbs the NF-kB, MAPK, or Apoptosis pathway to modulate ARG1 expression. In some embodiments, Pifithrin- μ perturbs the p53 signaling pathway to modulate ARG1 expression. In some embodiments, PDGF perturbs the PDGFR signaling pathway to modulate ARG1 expression. In some embodiments, Pacritinib (SB1518) perturbs the JAK/STAT signaling pathway to modulate ARG1 expression. In some embodiments, GDF2 (BMP9) perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, Crenolanib perturbs the PDGFR signaling pathway to modulate ARG1 expression. In some embodiments, prednisone perturbs the GR signaling pathway to modulate ARG1 expression. In some embodiments, HGF/SF perturbs the c-MET signaling pathway to modulate ARG1 expression. In some embodiments, Momelotinib perturbs the JAK/STAT signaling pathway to modulate ARG1 expression. In some embodiments, EGF perturbs the EGFR signaling pathway to modulate ARG1 expression. In some embodiments, Corticosterone perturbs the Mineralcorticoid receptor signaling pathway to modulate ARG1 expression. In some embodiments, FGF perturbs the FGFR signaling pathway to modulate ARG1 expression. In some embodiments, Thalidomide perturbs the NF-kB signaling pathway to modulate ARG1 expression. In some embodiments, Phenformin perturbs the AMPK signaling pathway to modulate ARG1 expression. In some embodiments, Tivozanib perturbs the Protein Tyrosine Kinase/RTK signaling pathway to modulate ARG1 expression. In some embodiments, BAY 87-2243 perturbs the Hypoxia activated signaling pathway to modulate ARG1 expression. In some embodiments, GZD824 Dimesylate perturbs the ABL signaling pathway to modulate ARG1 expression. In some embodiments, GDF10 (BMP3b) perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, PND-1186 perturbs the FAK signaling pathway to modulate ARG1 expression. In some embodiments, FRAX597 perturbs the PAK signaling pathway to modulate ARG1 expression. In some embodiments, BMP2 perturbs the TGF-B signaling pathway to modulate ARG1 expression. In some embodiments, Oligomycin A perturbs the ATP channel signaling pathway to modulate ARG1 expression. In some embodiments, Rifampicin perturbs the PXR signaling pathway to modulate ARG1 expression. In

some embodiments, MK-0752 perturbs the NOTCH signaling pathway to modulate ARG1 expression.

[00213] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the ARG1 gene. The ARG1 gene has a cytogenetic location of 6q23.2 and the genomic coordinate are on Chromosome 6 on the forward strand at position 131,573,144-131,584,332. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of ARG1. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, and p300. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, HNF1A, and MYC. The signaling proteins may include but are not limited to HIF1a, ESRA, NR3C1, JUN, RXR, STAT3, NR1H1, SMAD2/3, STAT1, and TEAD1.

[0013] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of SLC25A15. Compounds that may be used to modulate the SLC25A15 expression include, but are not limited to, Dasatinib, FRAX597, Merestinib, R788 (fostamatinib disodium hexahydrate), Bosutinib, bms-986094 (inx-189), Epinephrine, GDF2 (BMP9), Echinomycin, Corticosterone, IGF-1, CP-673451, GZD824 Dimesylate, EW-7197, PDGF, Wnt3a, and derivatives or analogs thereof. In some embodiments, Dasatinib perturbs the ABL signaling pathway to modulate SLC25A15 expression. In some embodiments, FRAX597 perturbs the PAK signaling pathway to modulate SLC25A15 expression. In some embodiments, Merestinib perturbs the c-MET signaling pathway to modulate SLC25A15 expression. In some embodiments, R788 (fostamatinib disodium hexahydrate) perturbs the Protein Tyrosine Kinase/RTK signaling pathway to modulate SLC25A15 expression. In some embodiments, Bosutinib perturbs the Src signaling pathway to modulate SLC25A15 expression. In some embodiments, Epinephrine perturbs the Adrenergic receptor signaling pathway to modulate SLC25A15 expression. In some embodiments, GDF2 (BMP9) perturbs the TGF-B signaling pathway to modulate SLC25A15 expression. In some embodiments, Echinomycin perturbs the Hypoxia activated signaling pathway to modulate SLC25A15 expression. In some embodiments, Corticosterone perturbs the Mineralcorticoid receptor signaling pathway to modulate SLC25A15 expression. In some embodiments, IGF-1 perturbs the IGF-1R/InsR signaling pathway to modulate SLC25A15 expression. In some embodiments, CP-673451 perturbs the PDGFR signaling pathway to modulate SLC25A15 expression. In some embodiments, GZD824 Dimesylate perturbs the ABL signaling pathway to

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modulate SLC25A15 expression. In some embodiments, EW-7197 perturbs the TGF- β signaling pathway to modulate SLC25A15 expression. In some embodiments, PDGF perturbs the PDGFR signaling pathway to modulate SLC25A15 expression. In some embodiments, Wnt3a perturbs the WNT signaling pathway to modulate SLC25A15 expression.

[00214] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the SLC25A15 gene. SLC25A15 has a cytogenetic location of 13q14.11 and the genomic coordinate are on Chromosome 13 on the forward strand at position 40,789,412-40,810,111. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of SLC25A15. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, and BRD4. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ONECUT2, and YY1. The signaling proteins may include but are not limited to ESRA, Jun, RXR, NR1H1, NF- κ B, NR3C1, SMAD2/3, and TP53.

[00215] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of SLC25A13. Compounds that may be used to modulate the SLC25A13 expression include, but are not limited to, TFP, 17-AAG (Tanespimycin), and derivatives or analogs thereof. In some embodiments, TFP perturbs the Calmodulin signaling pathway to modulate SLC25A13 expression. In some embodiments, 17-AAG (Tanespimycin) perturbs the Cell Cycle/DNA Damage Metabolic Enzyme/Protease signaling pathway to modulate SLC25A13 expression.

[00216] In some embodiments, methods of the present invention involve altering the composition and/or the structure of the insulated neighborhood containing the SLC25A13 gene. SLC25A13 has a cytogenetic location of 7q21.3 and the genomic coordinate are on Chromosome 7 on the reverse strand at position 96,120,220-96,322,147. Any chromatin mark, chromatin-associated protein, transcription factor and/or signaling protein that is associated with the insulated neighborhood, and/or any regions within or near the insulated neighborhood, may be targeted or altered to change the composition and/or structure of the insulated neighborhood, thereby modulating the expression of SLC25A13. The chromatin marks and/or chromatin-associated proteins may include but are not limited to H3K27ac, BRD4, p300, and SMC1. The transcription factors may include but are not limited to FOXA2, HNF4A, ONECUT1, ATF5, ONECUT2, YY1, HNF1A, and MYC. The signaling proteins may include but are not limited to

TCF7L2, HIF1a, ESRA, NR3C1, JUN, RXR, STAT3, NR1H1, NF- κ B, SMAD2/3, STAT1, TEAD1, and TP53.

[00217] In some embodiments, compositions and methods of the present invention may be used to treat a urea cycle disorder by modulating the expression of multiple urea cycle-related genes. As a non-limiting example, methods of the present invention may be used to modulate the expression of any one of the following groups of genes: NAGS, CPS1, ASS1, ASL, OTC, ARG1, and SLC25A15; CPS1, ASS1, ASL, OTC, ARG1, and SLC25A15; ASS1, CPS1, NAGS, ARG1, and SLC25A15; CPS1, ASS1, ASL, ARG1, and SLC25A15; CPS1, ASS1, ASL, OTC, and ARG1; CPS1, ASS1, OTC, ARG1, and SLC25A15; NAGS, CPS1, ALS, OTC, and ARG1; ASS1, ASL, OTC, and ARG1; ASS1, CPS1, NAGS, and ARG1; CPS1, ASS1, ARG1, and SLC25A15; CPS1, ASS1, OTC, and ARG1; CPS1, OTC, ARG1, and SLC25A15; NAGS, CPS1, OTC, and ARG1; CPS1, ARG1, and SLC25A13; CPS1, ARG1, and SLC25A15; CPS1, ASL, and ARG1; CPS1, NAGS, and ARG1; CPS1, OTC, and ARG1; NAGS, ASS1, and OTC; OTC, NAGS, and ARG1. Compounds that may be used to modulate multiple urea cycle-related genes include, but are not limited to, Dasatinib, Echinomycin, CP-673451, GDF2 (BMP9), Bosutinib, Epinephrine, Pacritinib (SB1518), Amuvatinib, FRAX597, Thalidomide, Crenolanib, BMP2, Deoxycorticosterone, TNF- α , Wnt3a, PDGF, IGF-1, Activin, HGF/SF, 17-AAG (Tanespimycin), R788 (fostamatinib disodium hexahydrate), GZD824 Dimesylate, BAY 87-2243, prednisone, Nodal, Momelotinib, FGF, EGF, Anti mullerian hormone, INNO-206 (aldoxorubicin), and Pifithrin- μ .

[00218] In some embodiments, targeting multiple urea cycle-related genes may be accomplished by utilizing a combination of compounds that each specifically modulates a urea cycle-related gene. In some embodiments, targeting multiple urea cycle-related genes may be accomplished by utilizing a single compound that is capable of modulating multiple urea cycle-related genes.

[00219] In some embodiment, compounds of the present invention may be used in combination with other drugs, such as Sodium phenylbutyrate (BUPHENYL®), glycerol phenylbutyrate (RAVICTI®), and sodium benzoate, to treat a urea cycle disorder.

IV. FORMULATIONS AND DELIVERY

Pharmaceutical Compositions

[00220] According to the present invention the compositions may be prepared as pharmaceutical compositions. It will be understood that such compositions necessarily comprise one or more active ingredients and, most often, a pharmaceutically acceptable excipient.

[00221] Relative amounts of the active ingredient, a pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present

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disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[00222] In some embodiments, the pharmaceutical compositions described herein may comprise at least one payload. As a non-limiting example, the pharmaceutical compositions may contain 1, 2, 3, 4 or 5 payloads.

[00223] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, rats, birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00224] In some embodiments, compositions are administered to humans, human patients or subjects.

Formulations

[00225] Formulations of the present invention can include, without limitation, saline, liposomes, lipid nanoparticles, polymers, peptides, proteins, cells transfected with viral vectors (e.g., for transfer or transplantation into a subject) and combinations thereof.

[00226] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. As used herein the term “pharmaceutical composition” refers to compositions comprising at least one active ingredient and optionally one or more pharmaceutically acceptable excipients.

[00227] In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00228] Formulations of the compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or

more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00229] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00230] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

Excipients and Diluents

[00231] In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00232] Excipients, as used herein, include, but are not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference in its entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00233] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

Inactive Ingredients

[00234] In some embodiments, the pharmaceutical compositions formulations may comprise at least one inactive ingredient. As used herein, the term “inactive ingredient” refers to one or more agents that do not contribute to the activity of the active ingredient of the pharmaceutical composition included in formulations. In some embodiments, all, none or some of the inactive ingredients which may be used in the formulations of the present invention may be approved by the US Food and Drug Administration (FDA).

[00235] In one embodiment, the pharmaceutical compositions comprise at least one inactive ingredient such as, but not limited to, 1,2,6-Hexanetriol; 1,2-Dimyristoyl-Sn-Glycero-3-(Phospho-S-(1-Glycerol)); 1,2-Dimyristoyl-Sn-Glycero-3-Phosphocholine; 1,2-Dioleoyl-Sn-Glycero-3-Phosphocholine; 1,2-Dipalmitoyl-Sn-Glycero-3-(Phospho-Rac-(1-Glycerol)); 1,2-Distearoyl-Sn-Glycero-3-(Phospho-Rac-(1-Glycerol)); 1,2-Distearoyl-Sn-Glycero-3-Phosphocholine; 1-O-Tolylbiguanide; 2-Ethyl-1,6-Hexanediol; Acetic Acid; Acetic Acid, Glacial; Acetic Anhydride; Acetone; Acetone Sodium Bisulfite; Acetylated Lanolin Alcohols; Acetylated Monoglycerides; Acetylcysteine; Acetyltryptophan, DL-; Acrylates Copolymer; Acrylic Acid-Isooctyl Acrylate Copolymer; Acrylic Adhesive 788; Activated Charcoal; Adcote 72A103; Adhesive Tape; Adipic Acid; Aerotex Resin 3730; Alanine; Albumin Aggregated; Albumin Colloidal; Albumin Human; Alcohol; Alcohol, Dehydrated; Alcohol, Denatured; Alcohol, Diluted; Alfadex; Alginic Acid; Alkyl Ammonium Sulfonic Acid Betaine; Alkyl Aryl Sodium Sulfonate; Allantoin; Allyl .Alpha.-Ionone; Almond Oil; Alpha-Terpineol; Alpha-Tocopherol; Alpha-Tocopherol Acetate, DL-; Alpha-Tocopherol, DL-; Aluminum Acetate; Aluminum Chlorhydroxy Allantoinate; Aluminum Hydroxide; Aluminum Hydroxide - Sucrose, Hydrated; Aluminum Hydroxide Gel; Aluminum Hydroxide Gel F 500; Aluminum Hydroxide Gel F 5000; Aluminum Monostearate; Aluminum Oxide; Aluminum Polyester; Aluminum Silicate; Aluminum Starch Octenylsuccinate; Aluminum Stearate; Aluminum Subacetate; Aluminum Sulfate Anhydrous; Amerchol C; Amerchol-Cab; Aminomethylpropanol; Ammonia; Ammonia Solution; Ammonia Solution, Strong; Ammonium Acetate; Ammonium Hydroxide; Ammonium Lauryl Sulfate; Ammonium Nonoxynol-4 Sulfate; Ammonium Salt Of C-12-C-15 Linear Primary Alcohol Ethoxylate; Ammonium Sulfate; Ammonyx; Amphoteric-2; Amphoteric-9; Anethole; Anhydrous Citric Acid; Anhydrous Dextrose; Anhydrous Lactose;

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Anhydrous Trisodium Citrate; Aniseed Oil; Anoxid Sbn; Antifoam; Antipyrine; Apaflurane; Apricot Kernel Oil Peg-6 Esters; Aquaphor; Arginine; Arlacel; Ascorbic Acid; Ascorbyl Palmitate; Aspartic Acid; Balsam Peru; Barium Sulfate; Beeswax; Beeswax, Synthetic; Beheneth-10; Bentonite; Benzalkonium Chloride; Benzenesulfonic Acid; Benzethonium Chloride; Benzododecinium Bromide; Benzoic Acid; Benzyl Alcohol; Benzyl Benzoate; Benzyl Chloride; Betadex; Bibapcitide; Bismuth Subgallate; Boric Acid; Brocrinat; Butane; Butyl Alcohol; Butyl Ester Of Vinyl Methyl Ether/Maleic Anhydride Copolymer (125000 Mw); Butyl Stearate; Butylated Hydroxyanisole; Butylated Hydroxytoluene; Butylene Glycol; Butylparaben; Butyric Acid; C20-40 Pareth-24; Caffeine; Calcium; Calcium Carbonate; Calcium Chloride; Calcium Gluceptate; Calcium Hydroxide; Calcium Lactate; Calcobutrol; Caldiamide Sodium; Caloxetate Trisodium; Calteridol Calcium; Canada Balsam; Caprylic/Capric Triglyceride; Caprylic/Capric/Stearic Triglyceride; Captan; Captisol; Caramel; Carbomer 1342; Carbomer 1382; Carbomer 934; Carbomer 934p; Carbomer 940; Carbomer 941; Carbomer 980; Carbomer 981; Carbomer Homopolymer Type B (Allyl Pentaerythritol Crosslinked); Carbomer Homopolymer Type C (Allyl Pentaerythritol Crosslinked); Carbon Dioxide; Carboxy Vinyl Copolymer; Carboxymethylcellulose; Carboxymethylcellulose Sodium; Carboxypolymethylene; Carrageenan; Carrageenan Salt; Castor Oil; Cedar Leaf Oil; Cellulose; Cellulose, Microcrystalline; Cerasynt-Se; Ceresin; Cetareth-12; Cetareth-15; Cetareth-30; Cetearyl Alcohol/Cetareth-20; Cetearyl Ethylhexanoate; Ceteth-10; Ceteth-2; Ceteth-20; Ceteth-23; Cetostearyl Alcohol; Cetrimonium Chloride; Cetyl Alcohol; Cetyl Esters Wax; Cetyl Palmitate; Cetylpyridinium Chloride; Chlorobutanol; Chlorobutanol Hemihydrate; Chlorobutanol, Anhydrous; Chlorocresol; Chloroxylenol; Cholesterol; Choleth; Choleth-24; Citrate; Citric Acid; Citric Acid Monohydrate; Citric Acid, Hydrous; Cocamide Ether Sulfate; Cocamine Oxide; Coco Betaine; Coco Diethanolamide; Coco Monoethanolamide; Cocoa Butter; Coco-Glycerides; Coconut Oil; Coconut Oil, Hydrogenated; Coconut Oil/Palm Kernel Oil Glycerides, Hydrogenated; Cocoyl Caprylocaprate; Cola Nitida Seed Extract; Collagen; Coloring Suspension; Corn Oil; Cottonseed Oil; Cream Base; Creatine; Creatinine; Cresol; Croscarmellose Sodium; Crospovidone; Cupric Sulfate; Cupric Sulfate Anhydrous; Cyclomethicone; Cyclomethicone/Dimethicone Copolyol; Cysteine; Cysteine Hydrochloride; Cysteine Hydrochloride Anhydrous; Cysteine, DI-; D&C Red No. 28; D&C Red No. 33; D&C Red No. 36; D&C Red No. 39; D&C Yellow No. 10; Dalfampridine; Daubert 1-5 Pestr (Matte) 164z; Decyl Methyl Sulfoxide; Dehydag Wax Sx; Dehydroacetic Acid; Dehymuls E; Denatonium Benzoate; Deoxycholic Acid; Dextran; Dextran 40; Dextrin; Dextrose; Dextrose Monohydrate; Dextrose Solution; Diatrizoic Acid; Diazolidinyl Urea; Dichlorobenzyl Alcohol; Dichlorodifluoromethane; Dichlorotetrafluoroethane; Diethanolamine; Diethyl Pyrocarbonate;

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Diethyl Sebacate; Diethylene Glycol Monoethyl Ether; Diethylhexyl Phthalate;
 Dihydroxyaluminum Aminoacetate; Diisopropanolamine; Diisopropyl Adipate; Diisopropyl
 Dilinoleate; Dimethicone 350; Dimethicone Copolyol; Dimethicone Mdx4-4210; Dimethicone
 Medical Fluid 360; Dimethyl Isosorbide; Dimethyl Sulfoxide; Dimethylaminoethyl
 Methacrylate-Butyl Methacrylate - Methyl Methacrylate Copolymer;
 Dimethyldioctadecylammonium Bentonite; Dimethylsiloxane/Methylvinylsiloxane Copolymer;
 Dinoseb Ammonium Salt; Dipalmitoylphosphatidylglycerol, DL-; Dipropylene Glycol; Disodium
 Cocoamphodiacetate; Disodium Laureth Sulfosuccinate; Disodium Lauryl Sulfosuccinate;
 Disodium Sulfosalicylate; Disofenin; Divinylbenzene Styrene Copolymer; Dmdm Hydantoin;
 Docosanol; Docusate Sodium; Duro-Tak 280-2516; Duro-Tak 387-2516; Duro-Tak 80-1196;
 Duro-Tak 87-2070; Duro-Tak 87-2194; Duro-Tak 87-2287; Duro-Tak 87-2296; Duro-Tak 87-
 2888; Duro-Tak 87-2979; Edetate Calcium Disodium; Edetate Disodium; Edetate Disodium
 Anhydrous; Edetate Sodium; Edetic Acid; Egg Phospholipids; Entsufof; Entsufof Sodium;
 Epilactose; Epiritetracycline Hydrochloride; Essence Bouquet 9200; Ethanolamine Hydrochloride;
 Ethyl Acetate; Ethyl Oleate; Ethylcelluloses; Ethylene Glycol; Ethylene Vinyl Acetate
 Copolymer; Ethylenediamine; Ethylenediamine Dihydrochloride; Ethylene-Propylene
 Copolymer; Ethylene-Vinyl Acetate Copolymer (28% Vinyl Acetate); Ethylene-Vinyl Acetate
 Copolymer (9% Vinylacetate); Ethylhexyl Hydroxystearate; Ethylparaben; Eucalyptol;
 Exametazime; Fat, Edible; Fat, Hard; Fatty Acid Esters; Fatty Acid Pentaerythriol Ester; Fatty
 Acids; Fatty Alcohol Citrate; Fatty Alcohols; Fd&C Blue No. 1; Fd&C Green No. 3; Fd&C Red
 No. 4; Fd&C Red No. 40; Fd&C Yellow No. 10 (Delisted); Fd&C Yellow No. 5; Fd&C Yellow
 No. 6; Ferric Chloride; Ferric Oxide; Flavor 89-186; Flavor 89-259; Flavor Df-119; Flavor Df-
 1530; Flavor Enhancer; Flavor Fig 827118; Flavor Raspberry Pfc-8407; Flavor Rhodia
 Pharmaceutical No. Rf 451; Fluorochlorohydrocarbons; Formaldehyde; Formaldehyde Solution;
 Fractionated Coconut Oil; Fragrance 3949-5; Fragrance 520a; Fragrance 6.007; Fragrance 91-
 122; Fragrance 9128-Y; Fragrance 93498g; Fragrance Balsam Pine No. 5124; Fragrance Bouquet
 10328; Fragrance Chemoderm 6401-B; Fragrance Chemoderm 6411; Fragrance Cream No.
 73457; Fragrance Cs-28197; Fragrance Felton 066m; Fragrance Firmenich 47373; Fragrance
 Givaudan Ess 9090/1c; Fragrance H-6540; Fragrance Herbal 10396; Fragrance Nj-1085;
 Fragrance P O Fl-147; Fragrance Pa 52805; Fragrance Pera Derm D; Fragrance Rbd-9819;
 Fragrance Shaw Mudge U-7776; Fragrance Tf 044078; Fragrance Ungerer Honeysuckle K 2771;
 Fragrance Ungerer N5195; Fructose; Gadolinium Oxide; Galactose; Gamma Cyclodextrin;
 Gelatin; Gelatin, Crosslinked; Gelfoam Sponge; Gellan Gum (Low Acyl); Gelva 737; Gentisic
 Acid; Gentisic Acid Ethanolamide; Gluceptate Sodium; Gluceptate Sodium Dihydrate;
 Gluconolactone; Glucuronic Acid; Glutamic Acid, DL-; Glutathione; Glycerin; Glycerol Ester Of

Hydrogenated Rosin; Glyceryl Citrate; Glyceryl Isostearate; Glyceryl Laurate; Glyceryl
 Monostearate; Glyceryl Oleate; Glyceryl Oleate/Propylene Glycol; Glyceryl Palmitate; Glyceryl
 Ricinoleate; Glyceryl Stearate; Glyceryl Stearate - Laureth-23; Glyceryl Stearate/Peg Stearate;
 Glyceryl Stearate/Peg-100 Stearate; Glyceryl Stearate/Peg-40 Stearate; Glyceryl Stearate-
 Stearamidoethyl Diethylamine; Glyceryl Trioleate; Glycine; Glycine Hydrochloride; Glycol
 Distearate; Glycol Stearate; Guanidine Hydrochloride; Guar Gum; Hair Conditioner (18n195-
 1m); Heptane; Hetastarch; Hexylene Glycol; High Density Polyethylene; Histidine; Human
 Albumin Microspheres; Hyaluronate Sodium; Hydrocarbon; Hydrocarbon Gel, Plasticized;
 Hydrochloric Acid; Hydrochloric Acid, Diluted; Hydrocortisone; Hydrogel Polymer; Hydrogen
 Peroxide; Hydrogenated Castor Oil; Hydrogenated Palm Oil; Hydrogenated Palm/Palm Kernel
 Oil Peg-6 Esters; Hydrogenated Polybutene 635-690; Hydroxide Ion; Hydroxyethyl Cellulose;
 Hydroxyethylpiperazine Ethane Sulfonic Acid; Hydroxymethyl Cellulose; Hydroxyoctacosanyl
 Hydroxystearate; Hydroxypropyl Cellulose; Hydroxypropyl Methylcellulose 2906;
 Hydroxypropyl-Beta-cyclodextrin; Hypromellose 2208 (15000 Mpa.S); Hypromellose 2910
 (15000 Mpa.S); Hypromelloses; Imidurea; Iodine; Iodoxamic Acid; Iofetamine Hydrochloride;
 Irish Moss Extract; Isobutane; Isoceteth-20; Isoleucine; Isooctyl Acrylate; Isopropyl Alcohol;
 Isopropyl Isostearate; Isopropyl Myristate; Isopropyl Myristate - Myristyl Alcohol; Isopropyl
 Palmitate; Isopropyl Stearate; Isostearic Acid; Isostearyl Alcohol; Isotonic Sodium Chloride
 Solution; Jelene; Kaolin; Kathon Cg; Kathon Cg II; Lactate; Lactic Acid; Lactic Acid, DL-;
 Lactic Acid, L-; Lactobionic Acid; Lactose; Lactose Monohydrate; Lactose, Hydrous; Laneth;
 Lanolin; Lanolin Alcohol - Mineral Oil; Lanolin Alcohols; Lanolin Anhydrous; Lanolin
 Cholesterols; Lanolin Nonionic Derivatives; Lanolin, Ethoxylated; Lanolin, Hydrogenated;
 Lauralkonium Chloride; Lauramine Oxide; Laurdimonium Hydrolyzed Animal Collagen;
 Laureth Sulfate; Laureth-2; Laureth-23; Laureth-4; Lauric Diethanolamide; Lauric Myristic
 Diethanolamide; Lauroyl Sarcosine; Lauryl Lactate; Lauryl Sulfate; Lavandula Angustifolia
 Flowering Top; Lecithin; Lecithin Unbleached; Lecithin, Egg; Lecithin, Hydrogenated; Lecithin,
 Hydrogenated Soy; Lecithin, Soybean; Lemon Oil; Leucine; Levulinic Acid; Lidofenin; Light
 Mineral Oil; Light Mineral Oil (85 Ssu); Limonene, (+/-)-; Lipocol Sc-15; Lysine; Lysine
 Acetate; Lysine Monohydrate; Magnesium Aluminum Silicate; Magnesium Aluminum Silicate
 Hydrate; Magnesium Chloride; Magnesium Nitrate; Magnesium Stearate; Maleic Acid;
 Mannitol; Maprofix; Mebrofenin; Medical Adhesive Modified S-15; Medical Antiform A-F
 Emulsion; Medronate Disodium; Medronic Acid; Meglumine; Menthol; Metacresol;
 Metaphosphoric Acid; Methanesulfonic Acid; Methionine; Methyl Alcohol; Methyl Gluceth-10;
 Methyl Gluceth-20; Methyl Gluceth-20 Sesquisteate; Methyl Glucose Sesquisteate; Methyl
 Laurate; Methyl Pyrrolidone; Methyl Salicylate; Methyl Stearate; Methylboronic Acid;

Methylcellulose (4000 Mpa.S); Methylcelluloses; Methylchloroisothiazolinone; Methylene Blue; Methylisothiazolinone; Methylparaben; Microcrystalline Wax; Mineral Oil; Mono And Diglyceride; Monostearyl Citrate; Monothioglycerol; Multisterol Extract; Myristyl Alcohol; Myristyl Lactate; Myristyl- γ -Picolinium Chloride; N-(Carbamoyl-Methoxy Peg-40)-1,2-Distearoyl-Cephalin Sodium; N,N-Dimethylacetamide; Niacinamide; Nioxime; Nitric Acid; Nitrogen; Nonoxynol Iodine; Nonoxynol-15; Nonoxynol-9; Norflurane; Oatmeal; Octadecene-1/Maleic Acid Copolymer; Octanoic Acid; Octisalate; Octoxynol-1; Octoxynol-40; Octoxynol-9; Octyldodecanol; Octylphenol Polymethylene; Oleic Acid; Oleth-10/Oleth-5; Oleth-2; Oleth-20; Oleyl Alcohol; Oleyl Oleate; Olive Oil; Oxidronate Disodium; Oxyquinoline; Palm Kernel Oil; Palmitamine Oxide; Parabens; Paraffin; Paraffin, White Soft; Parfum Creme 45/3; Peanut Oil; Peanut Oil, Refined; Pectin; Peg 6-32 Stearate/Glycol Stearate; Peg Vegetable Oil; Peg-100 Stearate; Peg-12 Glyceryl Laurate; Peg-120 Glyceryl Stearate; Peg-120 Methyl Glucose Dioleate; Peg-15 Cocamine; Peg-150 Distearate; Peg-2 Stearate; Peg-20 Sorbitan Isostearate; Peg-22 Methyl Ether/Dodecyl Glycol Copolymer; Peg-25 Propylene Glycol Stearate; Peg-4 Dilaurate; Peg-4 Laurate; Peg-40 Castor Oil; Peg-40 Sorbitan Diisostearate; Peg-45/Dodecyl Glycol Copolymer; Peg-5 Oleate; Peg-50 Stearate; Peg-54 Hydrogenated Castor Oil; Peg-6 Isostearate; Peg-60 Castor Oil; Peg-60 Hydrogenated Castor Oil; Peg-7 Methyl Ether; Peg-75 Lanolin; Peg-8 Laurate; Peg-8 Stearate; Pegoxol 7 Stearate; Pentadecalactone; Pentaerythritol Cocoate; Pentasodium Pentetate; Pentetate Calcium Trisodium; Pentetic Acid; Peppermint Oil; Perflutren; Perfume 25677; Perfume Bouquet; Perfume E-1991; Perfume Gd 5604; Perfume Tana 90/42 Scba; Perfume W-1952-1; Petrolatum; Petrolatum, White; Petroleum Distillates; Phenol; Phenol, Liquefied; Phenonip; Phenoxyethanol; Phenylalanine; Phenylethyl Alcohol; Phenylmercuric Acetate; Phenylmercuric Nitrate; Phosphatidyl Glycerol, Egg; Phospholipid; Phospholipid, Egg; Phospholipon 90g; Phosphoric Acid; Pine Needle Oil (*Pinus Sylvestris*); Piperazine Hexahydrate; Plastibase-50w; Polacrilin; Polidronium Chloride; Poloxamer 124; Poloxamer 181; Poloxamer 182; Poloxamer 188; Poloxamer 237; Poloxamer 407; Poly(Bis(P-Carboxyphenoxy)Propane Anhydride):Sebacic Acid; Poly(Dimethylsiloxane/Methylvinylsiloxane/Methylhydrogensiloxane) Dimethylvinyl Or Dimethylhydroxy Or Trimethyl Endblocked; Poly(DI-Lactic-Co-Glycolic Acid), (50:50; Poly(DI-Lactic-Co-Glycolic Acid), Ethyl Ester Terminated, (50:50; Polyacrylic Acid (250000 Mw); Polybutene (1400 Mw); Polycarbophil; Polyester; Polyester Polyamine Copolymer; Polyester Rayon; Polyethylene Glycol 1000; Polyethylene Glycol 1450; Polyethylene Glycol 1500; Polyethylene Glycol 1540; Polyethylene Glycol 200; Polyethylene Glycol 300; Polyethylene Glycol 300-1600; Polyethylene Glycol 3350; Polyethylene Glycol 400; Polyethylene Glycol 4000; Polyethylene Glycol 540; Polyethylene Glycol 600; Polyethylene

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Glycol 6000; Polyethylene Glycol 8000; Polyethylene Glycol 900; Polyethylene High Density Containing Ferric Oxide Black (<1%); Polyethylene Low Density Containing Barium Sulfate (20-24%); Polyethylene T; Polyethylene Terephthalates; Polyglactin; Polyglyceryl-3 Oleate; Polyglyceryl-4 Oleate; Polyhydroxyethyl Methacrylate; Polyisobutylene; Polyisobutylene (1100000 Mw); Polyisobutylene (35000 Mw); Polyisobutylene 178-236; Polyisobutylene 241-294; Polyisobutylene 35-39; Polyisobutylene Low Molecular Weight; Polyisobutylene Medium Molecular Weight; Polyisobutylene/Polybutene Adhesive; Polylactide; Polyols; Polyoxyethylene - Polyoxypropylene 1800; Polyoxyethylene Alcohols; Polyoxyethylene Fatty Acid Esters; Polyoxyethylene Propylene; Polyoxyl 20 Cetostearyl Ether; Polyoxyl 35 Castor Oil; Polyoxyl 40 Hydrogenated Castor Oil; Polyoxyl 40 Stearate; Polyoxyl 400 Stearate; Polyoxyl 6 And Polyoxyl 32 Palmitostearate; Polyoxyl Distearate; Polyoxyl Glyceryl Stearate; Polyoxyl Lanolin; Polyoxyl Palmitate; Polyoxyl Stearate; Polypropylene; Polypropylene Glycol; Polyquaternium-10; Polyquaternium-7 (70/30 Acrylamide/Dadmac; Polysiloxane; Polysorbate 20; Polysorbate 40; Polysorbate 60; Polysorbate 65; Polysorbate 80; Polyurethane; Polyvinyl Acetate; Polyvinyl Alcohol; Polyvinyl Chloride; Polyvinyl Chloride-Polyvinyl Acetate Copolymer; Polyvinylpyridine; Poppy Seed Oil; Potash; Potassium Acetate; Potassium Alum; Potassium Bicarbonate; Potassium Bisulfite; Potassium Chloride; Potassium Citrate; Potassium Hydroxide; Potassium Metabisulfite; Potassium Phosphate, Dibasic; Potassium Phosphate, Monobasic; Potassium Soap; Potassium Sorbate; Povidone Acrylate Copolymer; Povidone Hydrogel; Povidone K17; Povidone K25; Povidone K29/32; Povidone K30; Povidone K90; Povidone K90f; Povidone/Eicosene Copolymer; Povidones; Ppg-12/Smdi Copolymer; Ppg-15 Stearyl Ether; Ppg-20 Methyl Glucose Ether Distearate; Ppg-26 Oleate; Product Wat; Proline; Promulgen D; Promulgen G; Propane; Propellant A-46; Propyl Gallate; Propylene Carbonate; Propylene Glycol; Propylene Glycol Diacetate; Propylene Glycol Dicaprylate; Propylene Glycol Monolaurate; Propylene Glycol Monopalmitostearate; Propylene Glycol Palmitostearate; Propylene Glycol Ricinoleate; Propylene Glycol/Diazolidinyl Urea/Methylparaben/Propylparaben; Propylparaben; Protamine Sulfate; Protein Hydrolysate; Pvm/Ma Copolymer; Quaternium-15; Quaternium-15 Cis-Form; Quaternium-52; Ra-2397; Ra-3011; Saccharin; Saccharin Sodium; Saccharin Sodium Anhydrous; Safflower Oil; Sd Alcohol 3a; Sd Alcohol 40; Sd Alcohol 40-2; Sd Alcohol 40b; Sepineo P 600; Serine; Sesame Oil; Shea Butter; Silastic Brand Medical Grade Tubing; Silastic Medical Adhesive; Silicone Type A; Silica, Dental; Silicon; Silicon Dioxide; Silicon Dioxide, Colloidal; Silicone; Silicone Adhesive 4102; Silicone Adhesive 4502; Silicone Adhesive Bio-Psa Q7-4201; Silicone Adhesive Bio-Psa Q7-4301; Silicone Emulsion; Silicone/Polyester Film Strip; Simethicone; Simethicone Emulsion; Sipon Ls 20np; Soda Ash; Sodium Acetate; Sodium Acetate Anhydrous; Sodium Alkyl Sulfate;

Sodium Ascorbate; Sodium Benzoate; Sodium Bicarbonate; Sodium Bisulfate; Sodium Bisulfite; Sodium Borate; Sodium Borate Decahydrate; Sodium Carbonate; Sodium Carbonate Decahydrate; Sodium Carbonate Monohydrate; Sodium Cetostearyl Sulfate; Sodium Chlorate; Sodium Chloride; Sodium Chloride Injection; Sodium Chloride Injection, Bacteriostatic; Sodium Cholesteryl Sulfate; Sodium Citrate; Sodium Cocoyl Sarcosinate; Sodium Desoxycholate; Sodium Dithionite; Sodium Dodecylbenzenesulfonate; Sodium Formaldehyde Sulfoxylate; Sodium Gluconate; Sodium Hydroxide; Sodium Hypochlorite; Sodium Iodide; Sodium Lactate; Sodium Lactate, L-; Sodium Laureth-2 Sulfate; Sodium Laureth-3 Sulfate; Sodium Laureth-5 Sulfate; Sodium Lauroyl Sarcosinate; Sodium Lauryl Sulfate; Sodium Lauryl Sulfoacetate; Sodium Metabisulfite; Sodium Nitrate; Sodium Phosphate; Sodium Phosphate Dihydrate; Sodium Phosphate, Dibasic; Sodium Phosphate, Dibasic, Anhydrous; Sodium Phosphate, Dibasic, Dihydrate; Sodium Phosphate, Dibasic, Dodecahydrate; Sodium Phosphate, Dibasic, Heptahydrate; Sodium Phosphate, Monobasic; Sodium Phosphate, Monobasic, Anhydrous; Sodium Phosphate, Monobasic, Dihydrate; Sodium Phosphate, Monobasic, Monohydrate; Sodium Polyacrylate (2500000 Mw); Sodium Pyrophosphate; Sodium Pyrrolidone Carboxylate; Sodium Starch Glycolate; Sodium Succinate Hexahydrate; Sodium Sulfate; Sodium Sulfate Anhydrous; Sodium Sulfate Decahydrate; Sodium Sulfite; Sodium Sulfosuccinated Undecylenic Monoalkylolamide; Sodium Tartrate; Sodium Thioglycolate; Sodium Thiomalate; Sodium Thiosulfate; Sodium Thiosulfate Anhydrous; Sodium Trimetaphosphate; Sodium Xylenesulfonate; Somay 44; Sorbic Acid; Sorbitan; Sorbitan Isostearate; Sorbitan Monolaurate; Sorbitan Monooleate; Sorbitan Monopalmitate; Sorbitan Monostearate; Sorbitan Sesquioleate; Sorbitan Trioleate; Sorbitan Tristearate; Sorbitol; Sorbitol Solution; Soybean Flour; Soybean Oil; Spearmint Oil; Spermaceti; Squalane; Stabilized Oxychloro Complex; Stannous 2-Ethylhexanoate; Stannous Chloride; Stannous Chloride Anhydrous; Stannous Fluoride; Stannous Tartrate; Starch; Starch 1500, Pregelatinized; Starch, Corn; Stearalkonium Chloride; Stearalkonium Hectorite/Propylene Carbonate; Stearamidoethyl Diethylamine; Steareth-10; Steareth-100; Steareth-2; Steareth-20; Steareth-21; Steareth-40; Stearic Acid; Stearic Diethanolamide; Stearoxytrimethylsilane; Steartrimonium Hydrolyzed Animal Collagen; Stearyl Alcohol; Sterile Water For Inhalation; Styrene/Isoprene/Styrene Block Copolymer; Succimer; Succinic Acid; Sucralose; Sucrose; Sucrose Distearate; Sucrose Polyesters; Sulfacetamide Sodium; Sulfobutylether .Beta.-Cyclodextrin; Sulfur Dioxide; Sulfuric Acid; Sulfurous Acid; Surfactol Qs; Tagatose, D-; Talc; Tall Oil; Tallow Glycerides; Tartaric Acid; Tartaric Acid, DL-; Tenox; Tenox-2; Tert-Butyl Alcohol; Tert-Butyl Hydroperoxide; Tert-Butylhydroquinone; Tetrakis(2-Methoxyisobutylisocyanide)Copper(I) Tetrafluoroborate; Tetrapropyl Orthosilicate; Tetrafosmin; Theophylline; Thimerosal; Threonine; Thymol; Tin; Titanium Dioxide;

Tocopherol; Tocophersolan; Total parenteral nutrition, lipid emulsion; Triacetin; Tricaprylin; Trichloromonofluoromethane; Trideceth-10; Triethanolamine Lauryl Sulfate; Trifluoroacetic Acid; Triglycerides, Medium Chain; Trihydroxystearin; Trilaneth-4 Phosphate; Trilaureth-4 Phosphate; Trisodium Citrate Dihydrate; Trisodium Hedta; Triton 720; Triton X-200; Trolamine; Tromantadine; Tromethamine (TRIS); Tryptophan; Tyloxapol; Tyrosine; Undecylenic Acid; Union 76 Amsco-Res 6038; Urea; Valine; Vegetable Oil; Vegetable Oil Glyceride, Hydrogenated; Vegetable Oil, Hydrogenated; Versetamide; Viscarin; Viscose/Cotton; Vitamin E; Wax, Emulsifying; Wecobee Fs; White Ceresin Wax; White Wax; Xanthan Gum; Zinc; Zinc Acetate; Zinc Carbonate; Zinc Chloride; and Zinc Oxide.

[00236] Pharmaceutical composition formulations disclosed herein may include cations or anions. In one embodiment, the formulations include metal cations such as, but not limited to, Zn^{2+} , Ca^{2+} , Cu^{2+} , Mn^{2+} , Mg^{+} and combinations thereof. As a non-limiting example, formulations may include polymers and complexes with a metal cation (*See e.g.*, U.S. Pat. Nos. 6,265,389 and 6,555,525, each of which is herein incorporated by reference in its entirety).

[00237] Formulations of the invention may also include one or more pharmaceutically acceptable salts. As used herein, “pharmaceutically acceptable salts” refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, acetic acid, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzene sulfonic acid, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids.

[00238] Solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

V. ADMINISTRATION AND DOSING

Administration

[00239] The terms "administering" and "introducing" are used interchangeably herein and refer to the delivery of the pharmaceutical composition into a cell or a subject. In the case of delivery to a subject, the pharmaceutical composition is delivered by a method or route that results in at least partial localization of the introduced cells at a desired site, such as hepatocytes, such that a desired effect(s) is produced.

[00240] In one aspect of the method, the pharmaceutical composition may be administered via a route such as, but not limited to, enteral (into the intestine), gastroenteral, epidural (into the dura matter), oral (by way of the mouth), transdermal, peridural, intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intravenous bolus, intravenous drip, intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection (into a pathologic cavity) intracavitary (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), transvaginal, insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), in ear drops, auricular (in or by way of the ear), buccal (directed toward the cheek), conjunctival, cutaneous, dental (to a tooth or teeth), electro-osmosis, endocervical, endosinusial, endotracheal, extracorporeal, hemodialysis, infiltration, interstitial, intra-abdominal, intra-amniotic, intra-articular, intrabiliary, intrabronchial, intrabursal, intracartilaginous (within a cartilage), intracaudal (within the cauda equine), intracisternal (within the cisterna magna cerebellomedullaris), intracorneal (within the cornea), dental intracornal, intracoronary (within the coronary arteries), intracorporus cavernosum (within the dilatable spaces of the corporus

cavernosa of the penis), intradiscal (within a disc), intraductal (within a duct of a gland), intraduodenal (within the duodenum), intradural (within or beneath the dura), intraepidermal (to the epidermis), intraesophageal (to the esophagus), intragastric (within the stomach), intragingival (within the gingivae), intraileal (within the distal portion of the small intestine), intralesional (within or introduced directly to a localized lesion), intraluminal (within a lumen of a tube), intralymphatic (within the lymph), intramedullary (within the marrow cavity of a bone), intrameningeal (within the meninges), intramyocardial (within the myocardium), intraocular (within the eye), intraovarian (within the ovary), intrapericardial (within the pericardium), intrapleural (within the pleura), intraprostatic (within the prostate gland), intrapulmonary (within the lungs or its bronchi), intrasinal (within the nasal or periorbital sinuses), intraspinal (within the vertebral column), intrasynovial (within the synovial cavity of a joint), intratendinous (within a tendon), intratesticular (within the testicle), intrathecal (within the cerebrospinal fluid at any level of the cerebrospinal axis), intrathoracic (within the thorax), intratubular (within the tubules of an organ), intratumor (within a tumor), intratympanic (within the aurus media), intravascular (within a vessel or vessels), intraventricular (within a ventricle), iontophoresis (by means of electric current where ions of soluble salts migrate into the tissues of the body), irrigation (to bathe or flush open wounds or body cavities), laryngeal (directly upon the larynx), nasogastric (through the nose and into the stomach), occlusive dressing technique (topical route administration which is then covered by a dressing which occludes the area), ophthalmic (to the external eye), oropharyngeal (directly to the mouth and pharynx), parenteral, percutaneous, periarticular, peridural, perineural, periodontal, rectal, respiratory (within the respiratory tract by inhaling orally or nasally for local or systemic effect), retrobulbar (behind the pons or behind the eyeball), intramyocardial (entering the myocardium), soft tissue, subarachnoid, subconjunctival, submucosal, topical, transplacental (through or across the placenta), transtracheal (through the wall of the trachea), transtympanic (across or through the tympanic cavity), ureteral (to the ureter), urethral (to the urethra), vaginal, caudal block, diagnostic, nerve block, biliary perfusion, cardiac perfusion, photopheresis and spinal.

[00241] Modes of administration include injection, infusion, instillation, and/or ingestion.

"Injection" includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. In some examples, the route is intravenous. For the delivery of cells, administration by injection or infusion can be made.

[00242] In some embodiments, compounds of the present invention can be administered to cells systemically. The phrases "systemic administration," "administered systemically,"

"peripheral administration," and "administered peripherally" refer to the administration other than directly into a target site, tissue, or organ, such that it enters, instead, the subject's circulatory system and, thus, is subject to metabolism and other like processes. In other embodiments, compounds of the present invention can be administered to cells *ex vivo*, *i.e.*, the compounds can be administered to cells that have been removed from an organ or tissue and held outside the subject's body *e.g.*, in primary culture.

Dosing

[00243] The term "effective amount" refers to the amount of the active ingredient needed to prevent or alleviate at least one or more signs or symptoms of a specific disease and/or condition, and relates to a sufficient amount of a composition to provide the desired effect. The term "therapeutically effective amount" therefore refers to an amount of active ingredient or a composition comprising the active ingredient that is sufficient to promote a particular effect when administered to a typical subject. An effective amount would also include an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate "effective amount" can be determined by one of ordinary skill in the art using routine experimentation.

[00244] The pharmaceutical, diagnostic, or prophylactic compositions of the present invention may be administered to a subject using any amount and any route of administration effective for preventing, treating, managing, or diagnosing diseases, disorders and/or conditions. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. The subject may be a human, a mammal, or an animal. Compositions in accordance with the invention are typically formulated in unit dosage form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate diagnostic dose level for any particular individual will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific payload employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, and route of administration; the duration of the treatment; drugs used in combination or coincidental with the active ingredient; and like factors well known in the medical arts.

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[00245] In certain embodiments, pharmaceutical compositions in accordance with the present invention may be administered at dosage levels sufficient to deliver from about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 0.05 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, or prophylactic, effect.

[00246] The desired dosage of the composition present invention may be delivered only once, three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. As used herein, a “split dose” is the division of “single unit dose” or total daily dose into two or more doses, *e.g.*, two or more administrations of the “single unit dose”. As used herein, a “single unit dose” is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, *i.e.*, single administration event.

VI. DEFINITIONS

[00247] The term “analog”, as used herein, refers to a compound that is structurally related to the reference compound and shares a common functional activity with the reference compound.

[00248] The term “biologic”, as used herein, refers to a medical product made from a variety of natural sources such as micro-organism, plant, animal, or human cells.

[00249] The term “boundary”, as used herein, refers to a point, limit, or range indicating where a feature, element, or property ends or begins.

[00250] The term “compound”, as used herein, refers to a single agent or a pharmaceutically acceptable salt thereof, or a bioactive agent or drug.

[00251] The term “derivative”, as used herein, refers to a compound that differs in structure from the reference compound, but retains the essential properties of the reference molecule.

[00252] The term “downstream neighborhood gene”, as used herein, refers to a gene downstream of primary neighborhood gene that may be located within the same insulated neighborhood as the primary neighborhood gene.

[00253] The term “drug”, as used herein, refers to a substance other than food intended for use in the diagnosis, cure, alleviation, treatment, or prevention of disease and intended to affect the structure or any function of the body.

[00254] The term “enhancer”, as used herein, refers to regulatory DNA sequences that, when bound by transcription factors, enhance the transcription of an associated gene.

[00255] The term “gene”, as used herein, refers to a unit or segment of the genomic architecture of an organism, e.g., a chromosome. Genes may be coding or non-coding. Genes may be encoded as contiguous or non-contiguous polynucleotides. Genes may be DNA or RNA.

[00256] The term “genomic signaling center”, i.e., a “signaling center”, as used herein, refers to regions within insulated neighborhoods that include regions capable of binding context-specific combinatorial assemblies of signaling molecules/signaling proteins that participate in the regulation of the genes within that insulated neighborhood or among more than one insulated neighborhood.

[00257] The term “genomic system architecture”, as used herein, refers to the organization of an individual’s genome and includes chromosomes, topologically associating domains (TADs), and insulated neighborhoods.

[00258] The term “herbal preparation”, as used herein, refers to herbal medicines that contain parts of plants, or other plant materials, or combinations as active ingredients.

[00259] The term “insulated neighborhood” (IN), as used herein, refers to chromosome structure formed by the looping of two interacting sites in the chromosome sequence that may comprise CCCTC-Binding factor (CTCF) co-occupied by cohesin and affect the expression of genes in the insulated neighborhood as well as those genes in the vicinity of the insulated neighborhoods.

[00260] The term “insulator”, as used herein, refers to regulatory elements that block the ability of an enhancer to activate a gene when located between them and contribute to specific enhancer-gene interactions.

[00261] The term “master transcription factor”, as used herein, refers to signaling molecules which alter, whether to increase or decrease, the transcription of a target gene, e.g., a neighborhood gene and establish cell-type specific enhancers. Master transcription factors recruit additional signaling proteins, such as other transcription factors to enhancers to form signaling centers.

[00262] The term “minimal insulated neighborhood”, as used herein, refers to an insulated neighborhood having at least one neighborhood gene and associated regulatory sequence region or regions (RSRs) which facilitate the expression or repression of the neighborhood gene such as a promoter and/or enhancer and/or repressor regions, and the like.

[00263] The term “modulate”, as used herein, refers to an alteration (e.g., increase or decrease) in the expression of the target gene and/or activity of the gene product.

[00264] The term “neighborhood gene”, as used herein, refers to a gene localized within an insulated neighborhood.

[00265] The term “penetrance”, as used herein, refers to the proportion of individuals carrying a particular variant of a gene (e.g., mutation, allele or generally a genotype, whether wild type or not) that also exhibits an associated trait (phenotype) of that variant gene and in some situations is measured as the proportion of individuals with the mutation who exhibit clinical symptoms thus existing on a continuum.

[00266] The term “polypeptide”, as used herein, refers to a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances, the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long.

[00267] The term “primary neighborhood gene” as used herein, refers to a gene which is most commonly found within a specific insulated neighborhood along a chromosome.

[00268] The term “primary downstream boundary”, as used herein, refers to the insulated neighborhood boundary located downstream of a primary neighborhood gene.

[00269] The term “primary upstream boundary”, as used herein, refers to the insulated neighborhood boundary located upstream of a primary neighborhood gene.

[00270] The term “promoter” as used herein, refers to a DNA sequence that defines where transcription of a gene by RNA polymerase begins and defines the direction of transcription indicating which DNA strand will be transcribed.

[00271] The term “regulatory sequence regions”, as used herein, include but are not limited to regions, sections or zones along a chromosome whereby interactions with signaling molecules occur in order to alter expression of a neighborhood gene.

[00272] The term “repressor”, as used herein, refers to any protein that binds to DNA and therefore regulates the expression of genes by decreasing the rate of transcription.

[00273] The term “secondary downstream boundary”, as used herein, refers to the downstream boundary of a secondary loop within a primary insulated neighborhood.

[00274] The term “secondary upstream boundary”, as used herein, refers to the upstream boundary of a secondary loop within a primary insulated neighborhood.

[00275] The term “signaling center”, as used herein, refers to a defined region of a living organism that interacts with a defined set of biomolecules, such as signaling proteins or signaling molecules (e.g., transcription factors) to regulate gene expression in a context-specific manner.

[00276] The term “signaling molecule”, as used herein, refers to any entity, whether protein, nucleic acid (DNA or RNA), organic small molecule, lipid, sugar or other biomolecule, which interacts directly, or indirectly, with a regulatory sequence region on a chromosome.

[00277] The term “signaling transcription factor”, as used herein, refers to signaling molecules which alter, whether to increase or decrease, the transcription of a target gene, e.g., a neighborhood gene and also act as cell-cell signaling molecules.

[00278] The term “small molecule”, as used herein, refers to a low molecular weight drug, i.e. <5000 Daltons organic compound that may help regulate a biological process.

[00279] The terms “subject” and “patient” are used interchangeably herein and refer to an animal to whom treatment with the compositions according to the present invention is provided.

[00280] The term “super-enhancers”, as used herein, refers to are large clusters of transcriptional enhancers that drive expression of genes that define cell identity.

[00281] The term “therapeutic agent”, as used herein, refers to a substance that has the ability to cure a disease or ameliorate the symptoms of the disease.

[00282] The term “therapeutic or treatment outcome”, as used herein, refers to any result or effect (whether positive, negative or null) which arises as a consequence of the perturbation of a GSC or GSN. Examples of therapeutic outcomes include, but are not limited to, improvement or amelioration of the unwanted or negative conditions associated with a disease or disorder, lessening of side effects or symptoms, cure of a disease or disorder, or any improvement associated with the perturbation of a GSC or GSN.

[00283] The term “topologically associating domains” (TADs), as used herein, refers to structures that represent a modular organization of the chromatin and have boundaries that are shared by the different cell types of an organism.

[00284] The term “transcription factors”, as used herein, refers to signaling molecules which alter, whether to increase or decrease, the transcription of a target gene, e.g., a neighborhood gene.

[00285] The term “therapeutic or treatment liability”, as used herein, refers to a feature or characteristic associated with a treatment or treatment regime which is unwanted, harmful or which mitigates the therapies positive outcomes. Examples of treatment liabilities include for example toxicity, poor half-life, poor bioavailability, lack of or loss of efficacy or pharmacokinetic or pharmacodynamic risks.

[00286] The term “upstream neighborhood gene”, as used herein, refers to a gene upstream of a primary neighborhood gene that may be located within the same insulated neighborhood as the primary neighborhood gene.

[00287] The term “urea cycle disorder”, as used herein, refers to any disorder that is caused by a defect or malfunction in the urea cycle.

[00288] The term “urea cycle-related gene”, as used herein, refers to a gene whose gene product (e.g., RNA or protein) is involved in the urea cycle.

[00289] Described herein are compositions and methods for perturbation of genomic signaling centers (GSCs) or entire gene signaling networks (GSNs) for the treatment of urea cycle disorders (e.g., OTC deficiency). The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred materials and methods are now described. Other features, objects and advantages of the invention will be apparent from the description. In the description, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present description will control.

[00290] The present invention is further illustrated by the following non-limiting examples.

VII. EXAMPLES

Example 1. Experimental procedures

A. Hepatocyte cell culture

[00291] Cryopreserved hepatocytes were cultured in plating media for 16 hours, transferred to maintenance media for 4 hours. Cultured on serum-free media for 2 hours, then a compound was added. The hepatocytes were maintained on the serum-free media for 16 hours prior to gene expression analysis. Primary Human Hepatocytes were stored in the vapor phase of a liquid nitrogen freezer (about -130°C).

[00292] To seed the primary human hepatocytes, vials of cells were retrieved from the LN₂ freezer, thawed in a 37°C water bath, and swirled gently until only a sliver of ice remained. Using a 10ml serological pipet, cells were gently pipetted out of the vial and gently pipetted down the side of 50mL conical tube containing 20mL cold thaw medium. The vial was rinsed with about 1mL of thaw medium, and the rinse was added to the conical tube. Up to 2 vials may be added to one tube of 20mL thaw medium.

[00293] The conical tube(s) were gently inverted 2-3 times and centrifuged at 100 g for 10 minutes at 4°C with reduced braking (e.g. 4 out of 9). The thaw medium slowly was slowly aspirated to avoid the pellet. 4mL cold plating medium was added slowly down the side (8mL if combined 2 vials to 1 tube), and the vial was inverted gently several times to resuspend cells.

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[00294] Cells were kept on ice until 100µl of well-mixed cells were added to 400µl diluted Trypan blue and mixed by gentle inversion. They were counted using a hemocytometer (or Cellometer), and viability and viable cells/mL were noted. Cells were diluted to a desired concentration and seeded on collagen I-coated plates. Cells were pipetted slowly and gently onto plate, only 1-2 wells at a time. The remaining cells were mixed in the tubes frequently by gentle inversion. Cells were seeded at about 8.5×10^6 cells per plate in 6mL cold plating medium (10cm). Alternatively, 1.5×10^6 cells per well for a 6-well plate (1mL medium/well); 7×10^5 cells per well for 12-well plate (0.5mL/well); or 3.75×10^5 cells per well for a 24-well plate (0.5mL/well)

[00295] After all cells and medium were added to the plate, the plate was transferred to an incubator (37°C, 5% CO₂, about 90% humidity) and rocked forwards and backwards, then side to side several times each to distribute cells evenly across the plate or wells. The plate(s) were rocked again every 15 minutes for the first hour post-plating. About 4 hours post-plating (or first thing the morning if cells were plated in the evening), cells were washed once with PBS and complete maintenance medium was added. The primary human hepatocytes were maintained in the maintenance medium and transferred to fresh medium daily.

B. Starvation and compound treatment of hepatocytes

[00296] Two to three hours before treatment, cells cultured as described above were washed with PBS and the medium was changed to either: fresh maintenance medium (complete) or modified maintenance medium 4b.

[00297] Compound stocks were prepared at 1000x final concentration and added in a 2-step dilution to the medium to reduce risk of a compound precipitating out of solution when added to the cells, and to ensure reasonable pipetting volumes. One at a time, each compound was first diluted 10-fold in warm (about 37°C) modified maintenance medium (initial dilution = ID), mixed by vortexing, and the ID was diluted 100-fold into the cell culture (e.g. 5.1µl into 1 well of a 24-well plate containing 0.5mL medium). The plate was mixed by carefully swirling and after all wells were treated and returned to the incubator overnight. If desired, separate plates/wells were treated with vehicle-only controls and/or positive controls. If using multi-well plates, controls were included on each plate. After about 18 hours, cells were harvested for further analysis, e.g., ChIP-seq, RNA-seq, ATAC-seq, etc.

C. Media composition

[00298] The thaw medium contained 6mL isotonic percoll and 14mL high glucose DMEM (Invitrogen #11965 or similar). The plating medium contained 100mL Williams E medium (Invitrogen #A1217601, without phenol red) and the supplement pack #CM3000 from ThermoFisher Plating medium containing 5mL FBS, 10µl dexamethasone, and 3.6mL

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plating/maintenance cocktail. Stock trypan blue (0.4%, Invitrogen #15250) was diluted 1:5 in PBS.

[00299] The ThermoFisher complete maintenance medium contained supplement pack #CM4000 (1 μ l dexamethasone and 4mL maintenance cocktail) and 100mL Williams E (Invitrogen #A1217601, without phenol red).

[00300] The modified maintenance media had no stimulating factors (dexamethasone, insulin, etc.), and contained 100mL Williams E (Invitrogen #A1217601, without phenol red), 1mL L-Glutamine (Sigma #G7513) to 2mM, 1.5mL HEPES (VWR #J848) to 15mM, and 0.5mL penicillin/streptomycin (Invitrogen #15140) to a final concentration of 50U/mL each.

D. DNA purification

[00301] DNA purification was conducted as described in Ji et al., *PNAS* 112(12):3841-3846 (2015) Supporting Information, which is hereby incorporated by reference in its entirety. One milliliter of 2.5 M glycine was added to each plate of fixed cells and incubated for 5 minutes to quench the formaldehyde. The cells were washed twice with PBS. The cells were pelleted at 1,300 g for 5 minutes at 4°C. Then, 4×10^7 cells were collected in each tube. The cells were lysed gently with 1 mL of ice-cold Nonidet P-40 lysis buffer containing protease inhibitor on ice for 5 minutes (buffer recipes are provided below). The cell lysate was layered on top of 2.5 volumes of sucrose cushion made up of 24% (wt/vol) sucrose in Nonidet P-40 lysis buffer. This sample was centrifuged at 18,000 g for 10 minutes at 4°C to isolate the nuclei pellet (the supernatant represented the cytoplasmic fraction). The nuclei pellet was washed once with PBS/1 mM EDTA. The nuclei pellet was resuspended gently with 0.5mL glycerol buffer followed by incubation for 2 minutes on ice with an equal volume of nuclei lysis buffer. The sample was centrifuged at 16,000 g for 2 minutes at 4°C to isolate the chromatin pellet (the supernatant represented the nuclear soluble fraction). The chromatin pellet was washed twice with PBS/1 mM EDTA. The chromatin pellet was stored at - 80°C.

[00302] The Nonidet P-40 lysis buffer contained 10 mM Tris·HCl (pH 7.5), 150 mM NaCl, and 0.05% Nonidet P-40. The glycerol buffer contained 20 mM Tris·HCl (pH 7.9), 75 mM NaCl, 0.5 mM EDTA, 0.85 mM DTT, and 50% (vol/vol) glycerol. The nuclei lysis buffer contained 10 mM Hepes (pH 7.6), 1 mM DTT, 7.5 mM MgCl₂, 0.2 mM EDTA, 0.3 M NaCl, 1 M urea, and 1% Nonidet P-40.

E. Chromatin immunoprecipitation sequencing (ChIP-seq)

[00303] ChIP-seq was performed using the following protocol for primary hepatocytes and HepG2 cells to determine the composition and confirm the location of signaling centers.

i. Cell cross-linking

[00304] 2×10^7 cells were used for each run of ChIP-seq. Two ml of fresh 11% formaldehyde (FA) solution was added to 20ml media on 15cm plates to reach a 1.1% final concentration.

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Plates were swirled briefly and incubated at room temperature (RT) for 15 minutes. At the end of incubation, the FA was quenched by adding 1ml of 2.5M Glycine to plates and incubating for 5 minutes at RT. The media was discarded to a 1L beaker, and cells were washed twice with 20ml ice-cold PBS. PBS (10ml) was added to plates, and cells were scraped off the plate. The cells were transferred to 15ml conical tubes, and the tubes were placed on ice. Plates were washed with an additional 4ml of PBS and combined with cells in 15ml tubes. Tubes were centrifuged for 5 minutes at 1,500 rpm at 4°C in a tabletop centrifuge. PBS was aspirated, and the cells were flash frozen in liquid nitrogen. Pellets were stored at -80°C until ready to use.

ii. Pre-block magnetic beads

[00305] Thirty µl Protein G beads (per reaction) were added to a 1.5ml Protein LoBind Eppendorf tube. The beads were collected by magnet separation at RT for 30 seconds. Beads were washed 3 times with 1ml of blocking solution by incubating beads on a rotator at 4°C for 10 minutes and collecting the beads with the magnet. Five µg of an antibody was added to the 250µl of beads in block solution. The mix was transferred to a clean tube, and rotated overnight at 4°C. On the next day, buffer containing antibodies was removed, and beads were washed 3 times with 1.1ml blocking solution by incubating beads on a rotator at 4°C for 10 minutes and collecting the beads with the magnet. Beads were resuspended in 50µl of block solution and kept on ice until ready to use.

iii. Cell lysis, genomic fragmentation, and chromatin immunoprecipitation

[00306] COMPLETE® protease inhibitor cocktail was added to lysis buffer 1 (LB1) before use. One tablet was dissolved in 1ml of H₂O for a 50x solution. The cocktail was stored in aliquots at -20°C. Cells were resuspended in each tube in 8ml of LB1 and incubated on a rotator at 4°C for 10 minutes. Nuclei were spun down at 1,350 g for 5 minutes at 4°C. LB1 was aspirated, and cells were resuspended in each tube in 8ml of LB2 and incubated on a rotator at 4°C for 10 minutes.

[00307] A COVARIS® E220EVOLUTION™ ultrasonicator was programmed per the manufacturer's recommendations for high cell numbers. HepG2 cells were sonicated for 12 minutes, and primary hepatocyte samples were sonicated for 10 minutes. Lysates were transferred to clean 1.5ml Eppendorf tubes, and the tubes were centrifuged at 20,000 g for 10 minutes at 4°C to pellet debris. The supernatant was transferred to a 2ml Protein LoBind Eppendorf tube containing pre-blocked Protein G beads with pre-bound antibodies. Fifty µl of the supernatant was saved as input. Input material was kept at -80°C until ready to use. Tubes were rotated with beads overnight at 4°C.

iv. Wash, elution, and cross-link reversal

[00308] All washing steps were performed by rotating tubes for 5 minutes at 4°C. The beads were transferred to clean Protein LoBind Eppendorf tubes with every washing step. Beads were

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collected in 1.5ml Eppendorf tube using a magnet. Beads were washed twice with 1.1ml of sonication buffer. The magnetic stand was used to collect magnetic beads. Beads were washed twice with 1.1ml of wash buffer 2, and the magnetic stand was used again to collect magnetic beads. Beads were washed twice with 1.1ml of wash buffer 3. All residual Wash buffer 3 was removed, and beads were washed once with 1.1ml TE + 0.2% Triton X-100 buffer. Residual TE + 0.2% Triton X-100 buffer was removed, and beads were washed twice with TE buffer for 30 seconds each time. Residual TE buffer was removed, and beads were resuspended in 300µl of ChIP elution buffer. Two hundred fifty µl of ChIP elution buffer was added to 50µl of input, and the tubes were rotated with beads 1 hour at 65°C. Input sample was incubated overnight at 65°C oven without rotation. Tubes with beads were placed on a magnet, and the eluate was transferred to a fresh DNA LoBind Eppendorf tube. The eluate was incubated overnight at 65°C oven without rotation

v. Chromatin extraction and precipitation
[00309] Input and immunoprecipitant (IP) samples were transferred to fresh tubes, and 300µl of TE buffer was added to IP and Input samples to dilute SDS. RNase A (20mg/ml) was added to the tubes, and the tubes were incubated at 37°C for 30 minutes. Following incubation, 3µl of 1M CaCl₂ and 7µl of 20mg/ml Proteinase K were added, and incubated 1.5 hours at 55°C. MaXtract High Density 2ml gel tubes (Qiagen) were prepared by centrifugation at full speed for 30 seconds at RT. Six hundred µl of phenol/chloroform/isoamyl alcohol was added to each proteinase K reaction and transferred in about 1.2ml mixtures to the MaXtract tubes. Tubes were spun at 16,000 g for 5 minutes at RT. The aqueous phase was transferred to two clean DNA LoBind tubes (300µl in each tube), and 1.5µl glycogen, 30µl of 3M sodium acetate, and 900µl ethanol were added. The mixture was precipitated overnight at -20°C or for 1 hour at -80°C, and spun down at maximum speed for 20 minutes at 4°C. The ethanol was removed, and pellets were washed with 1ml of 75% ethanol by spinning tubes down at maximum speed for 5 minutes at 4°C. Remnants of ethanol were removed, and pellets were dried for 5 min at RT. Twenty-five µl of H₂O was added to each immunoprecipitant (IP) and input pellet, left standing for 5 minutes, and vortexed briefly. DNA from both tubes was combined to obtain 50µl of IP and 50µl of input DNA for each sample. One µl of this DNA was used to measure the amount of pulled down DNA using Qubit dsDNA HS assay (ThermoFisher, #Q32854). The total amount of immunoprecipitated material ranged from several ng (for TFs) to several hundred ng (for chromatin modifications). Six µl of DNA was analyzed using qRT-PCR to determine enrichment. The DNA was diluted if necessary. If enrichment was satisfactory, the rest was used for library preparation for DNA sequencing.

vi. Library preparation for DNA sequencing

[00310] Libraries were prepared using NEBNext Ultra II DNA library prep kit for Illumina (NEB, #E7645) using NEBNext Multiplex Oligos for Illumina (NEB, #6609S) according to manufacturer's instructions with the following modifications. The remaining ChIP sample (about 43µl) and 1µg of input samples for library preparations were brought up the volume of 50µl before the End Repair portion of the protocol. End Repair reactions were run in a PCR machine with a heated lid in a 96-well semi-skirted PCR plate (ThermoFisher, #AB1400) sealed with adhesive plate seals (ThermoFisher, #AB0558) leaving at least one empty well in-between different samples. Undiluted adapters were used for input samples, 1:10 diluted adapters for 5-100ng of ChIP material, and 1:25 diluted adapters for less than 5ng of ChIP material. Ligation reactions were run in a PCR machine with the heated lid off. Adapter ligated DNA was transferred to clean DNA LoBind Eppendorf tubes, and the volume was brought to 96.5µl using H₂O.

[00311] 200-600bp ChIP fragments were selected using SPRIselect magnetic beads (Beckman Coulter, #B23317). Thirty µl of the beads were added to 96.5µl of ChIP sample to bind fragments that are longer than 600 bp. The shorter fragments were transferred to a fresh DNA LoBind Eppendorf tube. Fifteen µl of beads were added to bind the DNA longer than 200bp, and beads were washed with DNA twice using freshly prepared 75% ethanol. DNA was eluted using 17µl of 0.1X TE buffer. About 15µl was collected.

[00312] Three µl of size-selected Input sample and all (15µl) of the ChIP sample was used for PCR. The amount of size-selected DNA was measured using a Qubit dsDNA HS assay. PCR was run for 7 cycles of for Input and ChIP samples with about 5-10ng of size-selected DNA, and 12 cycles with less than 5 ng of size-selected DNA. One-half of the PCR product (25µl) was purified with 22.5µl of AMPure XP beads (Beckman Coulter, #A63880) according to the manufacturer's instructions. PCR product was eluted with 17µl of 0.1X TE buffer, and the amount of PCT product was measured using Qubit dsDNA HS assay. An additional 4 cycles of PCR were run for the second half of samples with less than 5ng of PCR product, DNA was purified using 22.5µl of AMPure XP beads. The concentration was measured to determine whether there was an increased yield. Both halves were combined, and the volume was brought up to 50µl using H₂O.

[00313] A second round of purifications of DNA was run using 45µl of AMPure XP beads in 17µl of 0.1X TE, and the final yield was measured using Qubit dsDNA HS assay. This protocol produces from 20ng to 1mg of PCR product. The quality of the libraries was verified by diluting 1µl of each sample with H₂O if necessary using the High Sensitivity BioAnalyzer DNA kit (Agilent, #5067-4626) based on manufacturer's recommendations.

vii. Reagents

[00314] 11% Formaldehyde Solution (50mL) contained 14.9ml of 37% formaldehyde (final conc. 11%), 1 ml of 5M NaCl (final conc. 0.1 M), 100µl of 0.5M EDTA (pH 8) (final conc. 1mM), 50µl of 0.5M EGTA (pH 8) (final conc. 0.5mM), and 2.5 ml 1M Hepes (pH 7.5) (final conc. 50 mM).

[00315] Block Solution contained 0.5% BSA (w/v) in PBS and 500mg BSA in 100ml PBS. Block solution may be prepared up to about 4 days prior to use.

[00316] Lysis buffer 1 (LB1) (500ml) contained 25ml of 1 M Hepes-KOH, pH 7.5; 14ml of 5M NaCl; 1 ml of 0.5M EDTA, pH 8.0; 50ml of 100% Glycerol solution; 25ml of 10% NP-40; and 12.5ml of 10% Triton X-100. The pH was adjusted to 7.5. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00317] Lysis buffer 2 (LB2) (1000ml) contained 10ml of 1 M Tris-HCL, pH 8.0; 40ml of 5 M NaCl; 2ml of 0.5M EDTA, pH 8.0; and 2ml of 0.5M EGTA, pH 8.0. The pH was adjusted to 8.0. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00318] Sonication buffer (500ml) contained 25ml of 1M Hepes-KOH, pH 7.5; 14ml of 5M NaCl; 1ml of 0.5M EDTA, pH 8.0; 50ml of 10% Triton X-100; 10ml of 5% Na-deoxycholate; and 5ml of 10% SDS. The pH was adjusted to 7.5. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00319] Proteinase inhibitors were included in the LB1, LB2, and Sonication buffer.

[00320] Wash Buffer 2 (500ml) contained 25ml of 1M Hepes-KOH, pH 7.5; 35 ml of 5M NaCl; 1ml of 0.5M EDTA, pH 8.0; 50ml of 10% Triton X-100; 10ml of 5% Na-deoxycholate; and 5ml of 10% SDS. The pH was adjusted to 7.5. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00321] Wash Buffer 3 (500ml) contained 10ml of 1M Tris-HCL, pH 8.0; 1ml of 0.5M EDTA, pH 8.0; 125ml of 1M LiCl solution; 25ml of 10% NP-40; and 50ml of 5% Na-deoxycholate. The pH was adjusted to 8.0. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00322] ChIP elution Buffer (500ml) contained 25ml of 1 M Tris-HCL, pH 8.0; 10ml of 0.5M EDTA, pH 8.0; 50ml of 10% SDS; and 415ml of ddH₂O. The pH was adjusted to 7.5. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

F. Analysis of ChIP-seq results

[00323] All pass filter reads from each sample were trimmed of sequencing adapters using trim_galore 0.4.4 with default options. Trimmed reads were mapped against the human genome (assembly GRCh38/GCA_000001405.15 “no alt” analysis set merged with hs38d1/GCA_000786075.2) using bwa version 0.7.15 (Li (2013) arXiv:1303.3997v1) with

default parameters. Aligned read duplicates were assessed using picard 2.9.0 (<http://broadinstitute.github.io/picard>) and reads with a MAPQ<20 or those matching standard SAM flags 0x1804 were discarded. Standard QC were applied (read integrity, mapping statistics, library complexity, fragment bias) to remove unsatisfactory samples. Enriched ChIP-seq peaks were identified by comparing samples against whole cell extract controls using MACS2 version 2.1.0 (Zhang et al., Genome Biol. (2008) 9(9):R137), with significant peaks selected as those with an adjusted p-value < 0.01. Peaks overlapping known repetitive “blacklist” regions (ENCODE Project Consortium, Nature (2012) 489(7414:57-74) were discarded. ChIP-seq signals were also normalized by read depth and visualized using the UCSC browser.

G. RNA-seq

[00324] This protocol is a modified version of the following protocols: MagMAX *mir*Vana Total RNA Isolation Kit User Guide (Applied Biosystems #MAN0011131 Rev B.0), NEBNext Poly(A) mRNA Magnetic Isolation Module (E7490), and NEBNext Ultra Directional RNA Library Prep Kit for Illumina (E7420) (New England Biosystems #E74901).

[00325] The MagMAX *mir*Vana kit instructions (the section titled “Isolate RNA from cells” on pages 14-17) were used for isolation of total RNA from cells in culture. Two hundred µl of Lysis Binding Mix was used per well of the multiwell plate containing adherent cells (usually a 24-well plate).

[00326] For mRNA isolation and library prep, the NEBNext Poly(A) mRNA Magnetic Isolation Module and Directional Prep kit was used. RNA isolated from cells above was quantified, and prepared in 500µg of each sample in 50µl of nuclease-free water. This protocol may be run in microfuge tubes or in a 96-well plate.

[00327] The 80% ethanol was prepared fresh, and all elutions are done in 0.1X TE Buffer. For steps requiring Ampure XP beads, beads were at room temperature before use. Sample volumes were measured first and beads were pipetted. Section 1.9B (not 1.9A) was used for NEBNext Multiplex Oligos for Illumina (#E6609). Before starting the PCR enrichment, cDNA was quantified using the Qubit (DNA High Sensitivity Kit, ThermoFisher #Q32854). The PCR reaction was run for 12 cycles.

[00328] After purification of the PCR Reaction (Step 1.10), the libraries were quantified using the Qubit DNA High Sensitivity Kit. 1µl of each sample were diluted to 1-2ng/µl to run on the Bioanalyzer (DNA High Sensitivity Kit, Agilent # 5067-4626). If Bioanalyzer peaks were not clean (one narrow peak around 300bp), the AMPure XP bead cleanup step was repeated using a 0.9X or 1.0X beads:sample ratio. Then, the samples were quantified again with the Qubit, and run again on the Bioanalyzer (1-2ng/µl).

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[00329] Nuclear RNA from INTACT-purified nuclei or whole neocortical nuclei was converted to cDNA and amplified with the Nugen Ovation RNA-seq System V2. Libraries were sequenced using the Illumina HiSeq 2500.

H. RNA-seq data analysis

[00330] All pass filter reads from each sample were mapped against the human genome (assembly GRCh38/GCA_000001405.15 “no alt” analysis set merged with hs38d1/GCA_000786075.2) using two pass mapping via STAR version 2.5.3a (alignment parameters alignIntronMin=20; alignIntronMax=1000000; outFilterMismatchNmax=999; outFilterMismatchNoverLmax=0.05; outFilterType=BySJout; outFilterMultimapNmax=20; alignSJoverhangMin=8; alignSJDBoverhangMin=1; alignMatesGapMax=1000000) (Dobin et al., *Bioinformatics* (2012) 29(1):15-21). Genomic alignments were converted to transcriptome alignments based on reference transcript annotations from The Human GENCODE Gene Set release 24 (Harrow et al., *Genome Res.* (2012) 22(9): 1760-1774). Using unique and multimapped transcriptomic alignments, gene-level abundance estimates were computed using RSEM version 1.3.0 (Li and Dewey, *BMC Bioinformatics* (2011) 12:323) in a strand-aware manner, and including confidence interval sampling calculations, to arrive at posterior mean estimates (PME) of abundances (counts and normalized FPKM – fragments per kilobase of exon per million mapped fragments) from the underlying Bayesian model. Standard QC were applied (read integrity, mapping statistics, library complexity, fragment bias) to remove unsatisfactory samples. Differential gene expression was computed using the negative binomial model implemented by DESeq2 version 1.16.1 (Love et al., *Genome Biol.* (2014) 15(12):550). Log2 fold change and significance values were computed using PME count data (with replicates explicitly modeled versus pan-experiment controls), median ratio normalized, using maximum likelihood estimation rather than maximum a posteriori, and disabling the use of Cook’s distance cutoff when determining acceptable adjusted p-values. Significantly differential genes were assigned as those with an adjusted p-value < 0.01, a log2 fold change of ≥ 1 or ≤ -1 , and at least one replicate with PME FPKM ≥ 1 . RNA-seq signals were also normalized by read depth and visualized using the UCSC browser.

I. ATAC-seq

[00331] Hepatocytes were seeded overnight, then the serum and other factors were removed. After 2-3 hours, the cells were treated with the compound and incubated overnight. The cells were harvested and the nuclei were prepared for the transposition reaction. 50,000 bead bound nuclei were transposed using Tn5 transposase (Illumina FC-121-1030) as described in Mo et al., 2015, *Neuron* 86, 1369-1384, which is hereby incorporated by reference in its entirety. After 9-12 cycles of PCR amplification, libraries were sequenced on an Illumina HiSeq 2000. PCR was

performed using barcoded primers with extension at 72°C for 5 minutes, PCR, then the final PCR product was sequenced.

[00332] All obtained reads from each sample were trimmed using trim_galore 0.4.1 requiring Phred score ≥ 20 and read length ≥ 30 for data analysis. The trimmed reads were mapped against the human genome (hg19 build) using Bowtie2 (version 2.2.9) with the parameters: -t -q -N 1 -L 25 -X 2000 no-mixed no-discordant. All unmapped reads, non-uniquely mapped reads and PCR duplicates were removed. All the ATAC-seq peaks were called using MACS2 with the parameters --nolambda --nomodel -q 0.01 --SPMR. The ATAC-seq signal was visualized in the UCSC genome browser. ATAC-seq peaks that were at least 2 kb away from annotated promoters (RefSeq, Ensemble and UCSC Known Gene databases combined) were selected as distal ATAC-seq peaks.

J. qRT-PCR

[00333] qRT-PCR was performed as described in North et al., *PNAS*, 107(40) 17315-17320 (2010), which is hereby incorporated by reference in its entirety. qRT-PCR was performed with cDNA using the iQ5 Multicolor rtPCR Detection system from BioRad with 60°C annealing.

[00334] Analysis of the fold changes in expression as measured by qRT-PCR were performed using the technique below. The control was DMSO, and the treatment was the selected compound (CPD). The internal control was GAPDH or B-Actin, and the gene of interest is the target. First, the averages of the 4 conditions were calculated for normalization: DMSO:GAPDH, DMSO:Target, CPD: GAPDH, and CPD:Target. Next, the ΔCT of both control and treatment were calculated to normalize to internal control (GAPDH) using (DMSO:Target) - (DMSO:GAPDH) = ΔCT control and (CPD:Target) - (CPD: GAPDH) = ΔCT experimental. Then, the $\Delta\Delta CT$ was calculated by ΔCT experimental - ΔCT control. The Expression Fold Change was calculated by $2^{-(\Delta\Delta CT)}$ (2-fold expression change was shown by RNA-Seq results provided herein).

K. Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET)

[00335] ChIA-PET was performed as previously described in Chepelev et al. (2012) *Cell Res.* 22, 490-503; Fullwood et al. (2009) *Nature* 462, 58-64; Goh et al. (2012) *J. Vis. Exp.*, <http://dx.doi.org/10.3791/3770>; Li et al. (2012) *Cell* 148, 84-98; and Downen et al. (2014) *Cell* 159, 374-387, which are each hereby incorporated by reference in their entireties. Briefly, embryonic stem (ES) cells (up to 1×10^8 cells) were treated with 1% formaldehyde at room temperature for 20 minutes and then neutralized using 0.2M glycine. The crosslinked chromatin was fragmented by sonication to size lengths of 300-700 bp. The anti-SMC1 antibody (Bethyl, A300-055A) was used to enrich SMC1-bound chromatin fragments. A portion of ChIP DNA was eluted from antibody-coated beads for concentration quantification and for enrichment analysis

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using quantitative PCR. For ChIA-PET library construction ChIP DNA fragments were end-repaired using T4 DNA polymerase (NEB). ChIP DNA fragments were divided into two aliquots and either linker A or linker B was ligated to the fragment ends. The two linkers differ by two nucleotides which were used as a nucleotide barcode (Linker A with CG; Linker B with AT). After linker ligation, the two samples were combined and prepared for proximity ligation by diluting in a 20ml volume to minimize ligations between different DNA-protein complexes. The proximity ligation reaction was performed with T4 DNA ligase (Fermentas) and incubated without rocking at 22°C for 20 hours. During the proximity ligation DNA fragments with the same linker sequence were ligated within the same chromatin complex, which generated the ligation products with homodimeric linker composition. However, chimeric ligations between DNA fragments from different chromatin complexes could also occur, thus producing ligation products with heterodimeric linker composition. These heterodimeric linker products were used to assess the frequency of nonspecific ligations and were then removed.

i. DAY 1

[00336] The cells were crosslinked as described for ChIP. Frozen cell pellets were stored in the -80°C freezer until ready to use. This protocol requires at least 3×10^8 cells frozen in six 15ml Falcon tubes (50 million cells per tube). Six 100µl Protein G Dynabeads (for each ChIA-PET sample) was added to six 1.5ml Eppendorf tubes on ice. Beads were washed three times with 1.5 ml Block solution, and incubated end over end at 4°C for 10 minutes between each washing step to allow for efficient blocking. Protein G Dynabeads were resuspended in 250µl of Block solution in each of six tubes and 10µg of SMC1 antibody (Bethyl A300-055A) was added to each tube. The bead-antibody mixes were incubated at 4°C end-over-end overnight.

ii. DAY 2

[00337] Beads were washed three times with 1.5ml Block solution to remove unbound IgG and incubated end-over-end at 4°C for 10 minutes each time. Smc1-bound beads were resuspended in 100µl of Block solution and stored at 4 °C. Final lysis buffer 1 (8ml per sample) was prepared by adding 50x Protease inhibitor cocktail solution to Lysis buffer 1 (LB1) (1:50). Eight ml of Final lysis buffer 1 was added to each frozen cell pellet (8ml per sample x 6). The cells were thoroughly resuspended and thawed on ice by pipetting up and down. The cell suspension was incubated again end-over-end for 10 minutes at 4 °C. The suspension was centrifuged at 1,350 x g for 5 minutes at 4 °C. Concurrently, Final lysis buffer 2 (8ml per sample) was prepared by adding 50x Protease inhibitor cocktail solution to lysis buffer 2 (LB2) (1:50)

[00338] After centrifugation, the supernatant was discarded, and the nuclei were thoroughly resuspended in 8ml Final lysis buffer 2 by pipetting up and down. The cell suspension was incubated end-over-end for 10 minutes at 4°C. The suspension was centrifuged at 1,350 x g for 5 minutes at 4°C. During incubation and centrifugation, the Final sonication buffer (15ml per

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sample) was prepared by adding 50x Protease inhibitor cocktail solution to sonication buffer (1:50). The supernatant was discarded, and the nuclei were fully resuspended in 15ml Final sonication buffer by pipetting up and down. The nuclear extract was extracted to fifteen 1ml Covaris Evolution E220 sonication tubes on ice. An aliquot of 10µl was used to check the size of unsonicated chromatin on a gel.

[00339] A Covaris sonicator was programmed according to manufacturer's instructions (12 minutes per 20 million cells = 12x15= 3 hours). The samples were sequentially sequenced as described above. The goal is to break chromatin DNA to 200-600 bp. If sonication fragments are too big, false positives become more frequent. The sonicated nuclear extract was dispensed into 1.5ml Eppendorf tubes. 1.5ml samples are centrifuged at full speed at 4°C for 10 minutes. Supernatant (SNE) was pooled into a new pre-cooled 50ml Falcon tube, and brought to a volume of 18ml with sonication buffer. Two tubes of 50µl were taken as input and to check the size of fragments. 250µl of ChIP elution buffer was added and reverse crosslinking occurred at 65°C overnight in the oven. After reversal of crosslinking, the size of sonication fragments was determined on a gel.

[00340] Three ml of sonicated extract was added to 100 µl Protein G beads with SMC1 antibodies in each of six clean 15ml Falcon tubes. The tubes containing SNE-bead mix were incubated end-over-end at 4°C overnight (14 to 18 hours).

iii. DAY 3

[00341] Half the volume (1.5ml) of the SNE-bead mix was added to each of six pre-chilled tubes and SNE was removed using a magnet. The tubes were sequentially washed as follows: 1) 1.5ml of Sonication buffer was added, the beads were resuspended and rotated for 5 minutes at 4°C for binding, then the liquid was removed (step performed twice); 2) 1.5ml of high-salt sonication buffer was added, and the beads were resuspended and rotated for 5 minutes at 4°C for binding, then the liquid was removed (step performed twice); 3) 1.5ml of high-salt sonication buffer was added, and the beads were resuspended and rotated for 5 minutes at 4°C for binding, then the liquid was removed (step performed twice); 4) 1.5ml of LiCl buffer was added, and the cells were resuspended and incubated end-over-end for 5 minutes for binding, then the liquid was removed (step performed twice); 5) 1.5ml of 1X TE + 0.2% Triton X-100 was used to wash the cells for 5 minutes for binding, then the liquid was removed; and 1.5ml of ice-cold TE Buffer was used to wash the cells for 30 seconds for binding, then the liquid was removed (step performed twice). Beads from all six tubes were sequentially resuspended in beads in one 1,000ul tube of 1X ice-cold TE buffer.

[00342] ChIP-DNA was quantified using the following protocol. Ten percent of beads (by volume), or 100µl, were transferred into a new 1.5ml tube, using a magnet. Beads were resuspended in 300µl of ChIP elution buffer and the tube was rotated with beads for 1 hour at

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65°C. The tube with beads was placed on a magnet and the eluate was transferred to a fresh DNA LoBind Eppendorf tube. The eluate was incubated overnight at 65°C oven without rotating. Immuno-precipitated samples were transferred to fresh tubes, and 300µl of TE buffer was added to the immuno-precipitants and Input samples to dilute. Five µl of RNase A (20mg/ml) was added, and the tube was incubated at 37°C for 30 minutes.

[00343] Following incubation, 3µl of 1M CaCl₂ and 7µl of 20 mg/ml Proteinase K was added to the tube and incubated 1.5 hours at 55°C. MaXtract High Density 2ml gel tubes (Qiagen) were prepared by centrifuging them at full speed for 30 seconds at RT. 600µl of phenol/chloroform/isoamyl alcohol was added to each proteinase K reaction. About 1.2ml of the mixtures was transferred to the MaXtract tubes. Tubes were spun at 16,000 g for 5 minutes at RT. The aqueous phase was transferred to two clean DNA LoBind tubes (300µl in each tube), and 1µl glycogen, 30 µl of 3M sodium acetate, and 900µl ethanol was added. The mixture was allowed to precipitate overnight at -20°C or for 1 hour at -80°C.

[00344] The mixture was spun down at maximum speed for 20 minutes at 4°C, ethanol was removed, and the pellets were washed with 1ml of 75% ethanol by spinning tubes down at maximum speed for 5 minutes at 4°C. All remnants of ethanol were removed, and pellets were dried for 5 minutes at RT. H₂O was added to each tube. Each tube was allowed to stand for 5 minutes, and vortexed briefly. DNA from both tubes was combined to obtain 50µl of IP and 100µl of Input DNA.

[00345] The amount of DNA collected was quantitated by ChIP using Qubit (Invitrogen #Q32856). One µl intercalating dye was combined with each measure 1µl of sample. Two standards that come with the kit were used. DNA from only 10% of the beads was measured. About 400ng of chromatin in 900µl of bead suspension was obtained with a good enrichment at enhancers and promoters as measured by qPCR.

iv. DAY 3 or 4

[00346] End-blunting of ChIP-DNA was performed on the beads using the following protocol. The remaining chromatin/beads were split by pipetting, and 450µl of bead suspension was aliquoted into 2 tubes. Beads were collected on a magnet. Supernatant was removed, and then the beads were resuspended in the following reaction mix: 70µl 10X NEB buffer 2.1 (NEB, M0203L), 7µl 10mM dNTPs, 615.8µl dH₂O, and 7.2µl of 3U/µl T4 DNA Polymerase (NEB, M0203L). The beads were incubated at 37°C with rotation for 40 minutes. Beads were collected with a magnet, then the beads were washed 3 times with 1ml ice-cold ChIA-PET Wash Buffer (30 seconds per each wash).

[00347] On-Bead A-tailing was performed by preparing Klenow (3' to 5' exo-) master mix as stated below: 70µl 10X NEB buffer 2, 7µl 10mM dATP, 616µl dH₂O, and 7µl of 3U/µl Klenow (3' to 5' exo-) (NEB, M0212L). The mixture was incubated at 37°C with rotation for 50 minutes.

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Beads were collected with a magnet, then beads were washed 3 times with 1ml of ice-cold ChIA-PET Wash Buffer (30 seconds per each wash).

[00348] Linkers were thawed gently on ice. Linkers were mixed well with water gently by pipetting, then with PEG buffer, then gently vortexed. Then, 1394µl of master mix and 6µl of ligase was added per tube and mixed by inversion. Parafilm was put on the tube, and the tube was incubated at 16°C with rotation overnight (at least 16 hours). The biotinylated linker was ligated to ChIP-DNA on beads by setting up the following reaction mix and adding reagents in order: 1110µl dH₂O, 4µl 200ng/µl biotinylated bridge linker, 280µl 5X T4 DNA ligase buffer with PEG (Invitrogen), and 6µl 30 U/µl T4 DNA ligase (Fermentas).

v. DAY 5

[00349] Exonuclease lambda/Exonuclease I On-Bead digestion was performed using the following protocol. Beads were collected with a magnet and washed 3 times with 1ml of ice-cold ChIA-PET Wash Buffer (30 seconds per each wash). The Wash buffer was removed from beads, then resuspended in the following reaction mix: 70µl 10X lambda nuclease buffer (NEB, M0262L), 618µl nuclease-free dH₂O, 6µl 5 U/µl Lambda Exonuclease (NEB, M0262L), and 6µl Exonuclease I (NEB, M0293L). The reaction was incubated at 37°C with rotation for 1 hour. Beads were collected with a magnet, and beads were washed 3 times with 1ml ice-cold ChIA-PET Wash Buffer (30 seconds per each wash).

[00350] Chromatin complexes were eluted off the beads by removing all residual buffer and resuspending the beads in 300µl of ChIP elution buffer. The tube with beads was rotated 1 hour at 65°C. The tube was placed on a magnet and the eluate was transferred to a fresh DNA LoBind Eppendorf tube. The eluate was incubated overnight at 65°C in an oven without rotating.

vi. DAY 6

[00351] The eluted sample was transferred to a fresh tube and 300µl of TE buffer was added to dilute the SDS. Three µl of RNase A (30mg/ml) was added to the tube, and the mixture was incubated at 37°C for 30 minutes. Following incubation, 3µl of 1M CaCl₂ and 7µl of 20 mg/ml Proteinase K was added, and the tube was incubated again for 1.5 hours at 55°C. MaXtract High Density 2ml gel tubes (Qiagen) were precipitated by centrifuging them at full speed for 30 seconds at RT. Six hundred µl of phenol/chloroform/isoamyl alcohol was added to each proteinase K reaction, and about 1.2ml of the mixture was transferred to the MaXtract tubes. Tubes were spun at 16,000 x g for 5 minutes at RT.

[00352] The aqueous phase was transferred to two clean DNA LoBind tubes (300µl in each tube), and 1µl glycogen, 30µl of 3M sodium acetate, and 900µl ethanol is added. The mixture was precipitated for 1 hour at -80°C. The tubes were spun down at maximum speed for 30 minutes at 4°C, and the ethanol was removed. The pellets were washed with 1ml of 75% ethanol by spinning tubes down at maximum speed for 5 minutes at 4°C. Remnants of ethanol were

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removed, and the pellets were dried for 5 minutes at RT. Thirty μ l of H₂O was added to the pellet and allowed to stand for 5 minutes. The pellet mixture was vortexed briefly, and spun down to collect the DNA.

[00353] Qubit and DNA High Sensitivity ChIP were performed to quantify and assess the quality of proximity ligated DNA products. About 120 ng of the product was obtained.

vii. DAY 7

[00354] Components for Nextera tagmentation were then prepared. One hundred ng of DNA was divided into four 25 μ l reactions containing 12.5 μ l 2X Tagmentation buffer (Nextera), 1 μ l nuclease-free dH₂O, 2.5 μ l Tn5 enzyme (Nextera), and 9 μ l DNA (25ng). Fragments of each of the reactions were analyzed on a Bioanalyzer for quality control.

[00355] The reactions were incubated at 55°C for 5 minutes, then at 10°C for 10 minutes. Twenty-five μ l of H₂O was added, and tagmented DNA was purified using Zymo columns. Three hundred fifty μ l of Binding Buffer was added to the sample, and the mixture was loaded into a column and spun at 13,000 rpm for 30 seconds. The flow through was re-applied and the columns were spun again. The columns are washed twice with 200 μ l of wash buffer and spun for 1 minute to dry the membrane. The column was transferred to a clean Eppendorf tube and 25 μ l of Elution buffer was added. The tube was spun down for 1 minute. This step was repeated with another 25 μ l of elution buffer. All tagmented DNA was combined into one tube.

[00356] ChIA-PETs was immobilized on Streptavidin beads using the following steps. 2X B&W Buffer (40ml) was prepared as follows for coupling of nucleic acids: 400 μ l 1M Tris-HCl pH 8.0 (10mM final), 80 μ l 1M EDTA (1mM final), 16ml 5M NaCl (2M final), and 23.52ml dH₂O. 1X B&W Buffer (40ml total) was prepared by adding 20ml dH₂O to 20ml of the 2X B&W Buffer.

[00357] MyOne Streptavidin Dynabeads M-280 were allowed to come to room temperature for 30 minutes, and 30 μ l of beads were transferred to a new 1.5ml tube. Beads were washed with 150 μ l of 2X B&W Buffer twice. Beads were resuspended in 100 μ l of iBlock buffer (Applied Biosystems), and mixed. The mixture was incubated at RT for 45 minutes on a rotator.

[00358] I-BLOCK Reagent was prepared to contain: 0.2% I-Block reagent (0.2 g), 1X PBS or 1X TBS (10 ml 10X PBS or 10X TBS), 0.05% Tween-20 (50 μ l), and H₂O to 100ml. 10X PBS and I-BLOCK reagent was added to H₂O, and the mixture was microwaved for 40 seconds (not allowed to boil), then stirred. Tween-20 was added after the solution is cooled. The solution remained opaque, but particles dissolved. The solution was cooled to RT for use.

[00359] During incubation of beads, 500ng of sheared genomic DNA was added to 50 μ l of H₂O and 50 μ l of 2X B&W Buffer. When the beads finished incubating with the iBLOCK buffer, they were washed twice with 200 μ l of 1X B&W buffer. The wash buffer was discarded, and 100 μ l of the sheared genomic DNA was added. The mixture was incubated with rotation for 30

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minutes at RT. The beads were washed twice with 200µl of 1X B&W buffer. Tagmented DNA was added to the beads with an equal volume of 2X B&W buffer and incubated for 45 minutes at RT with rotation. The beads were washed 5 times with 500µl of 2xSSC/0.5% SDS buffer (30 seconds each time) followed by 2 washes with 500ml of 1X B&W Buffer and incubated each after wash for 5 minutes at RT with rotation. The beads were washed once with 100µl elution buffer (EB) from a Qiagen Kit by resuspending beads gently and putting the tube on a magnet. The supernatant was removed from the beads, and they were resuspended in 30µl of EB.

[00360] A paired end sequencing library was constructed on beads using the following protocol. Ten µl of beads are tested by PCR with 10 cycles of amplification. The 50µl of the PCR mixture contains: 10µl of bead DNA, 15µl NPM mix (from Illumina Nextera kit), 5µl of PPC PCR primer, 5µl of Index Primer 1 (i7), 5µl of Index Primer 2 (i5), and 10µl of H₂O. PCR was performed using the following cycle conditions: denaturing the DNA at 72°C for 3 minutes, then 10-12 cycles of 98°C for 10 seconds, 63°C for 30 seconds, and 72°C for 50 seconds, and a final extension of 72°C for 5 minutes. The number of cycles was adjusted to obtain about 300ng of DNA total with four 25 µl reactions. The PCR product may be held at 4°C for an indefinite amount of time.

[00361] The PCR product was cleaned-up using AMPure beads. Beads were allowed to come to RT for 30 minutes before using. Fifty µl of the PCR reaction was transferred to a new Low-Bind Tube and (1.8x volume) 90µl of AMPure beads was added. The mixture was pipetted well and incubated at RT for 5 minutes. A magnet was used for 3 minutes to collect beads and remove the supernatant. Three hundred µl of freshly prepared 80% ethanol was added to the beads on the magnet, and the ethanol was carefully discarded. The wash was repeated, and then all ethanol was removed. The beads were dried on the magnet rack for 10 minutes. Ten µl EB was added to the beads, mixed well, and incubated for 5 minutes at RT. The eluate was collected, and 1µl of eluate was used for Qubit and Bioanalyzer.

[00362] The library was cloned to verify complexity using the following protocol. One µl of the library was diluted at 1:10. A PCR reaction was performed as described below. Primers that anneal to Illumina adapters were chosen (T_m=52.2°C). The PCR reaction mixture (total volume: 50µl) contained the following: 10µl of 5X GoTaq buffer, 1µl of 10 mM dNTP, 5µl of 10µM primer mix, 0.25µl of GoTaq polymerase, 1µl of diluted template DNA, and 32.75µl of H₂O. PCR was performed using the following cycle conditions: denaturing the DNA at 95°C for 2 minutes and 20 cycles at the following conditions: 95°C for 60 seconds, 50°C for 60 seconds, and 72°C for 30 seconds with a final extension at 72°C for 5 minutes. The PCR product may be held at 4°C for an indefinite amount of time.

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[00363] The PCR product was ligated with the pGEM® T-Easy vector (Promega) protocol. Five µl of 2X T4 Quick ligase buffer, 1µl of pGEM® T-Easy vector, 1µl of T4 ligase, 1µl of PCR product, and 2µl of H₂O were combined to a total volume of 10µl. The product was incubated for 1 hour at RT and 2µl was used to transform Stellar competent cells. Two hundred µl of 500µl of cells were plated in SOC media. The next day, 20 colonies were selected for Sanger sequencing using a T7 promoter primer. 60% clones had a full adapter, and 15% had a partial adapter.

viii. Reagents

[00364] Protein G Dynabeads for 10 samples were from Invitrogen Dynal, Cat# 10003D. Block solution (50ml) contained 0.25g BSA dissolved in 50ml of ddH₂O (0.5% BSA, w/v), and was stored at 4°C for 2 days before use.

[00365] Lysis buffer 1 (LB1) (500ml) contained 25ml of 1M Hepes-KOH, pH 7.5; 14ml of 5M NaCl; 1ml of 0.5 M EDTA, pH 8.0; 50ml of 100% Glycerol solution; 25ml of 10% NP-40; and 12.5ml of 10% Triton X-100. The pH was adjusted to 7.5. The buffer was sterile-filtered, and stored at 4°C. The pH was re-checked immediately prior to use. Lysis buffer 2 (LB2) (1000ml) contained 10ml of 1M Tris-HCL, pH 8.0; 40ml of 5 M NaCl; 2ml of 0.5 M EDTA, pH 8.0; and 2ml of 0.5 M EGTA, pH 8.0. The pH was adjusted to 8.0. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00366] Sonication buffer (500ml) contained 25ml of 1M Hepes-KOH, pH 7.5; 14ml of 5M NaCl; 1ml of 0.5 M EDTA, pH 8.0; 50ml of 10% Triton X-100; 10ml of 5% Na-deoxycholate; and 5ml of 10% SDS. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use. High-salt sonication buffer (500ml) contained 25ml of 1M Hepes-KOH, pH 7.5; 35ml of 5M NaCl; 1ml of 0.5 M EDTA, pH 8.0; 50ml of 10% Triton X-100; 10ml of 5% Na-deoxycholate; and 5ml of 10% SDS. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00367] LiCl wash buffer (500 ml) contained 10ml of 1M Tris-HCL, pH 8.0; 1ml of 0.5M EDTA, pH 8.0; 125ml of 1M LiCl solution; 25ml of 10% NP-40; and 50ml of 5% Na-deoxycholate. The pH was adjusted to 8.0. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00368] Elution buffer (500ml) used to quantify the amount of ChIP DNA contained 25ml of 1M Tris-HCL, pH 8.0; 10ml of 0.5M EDTA, pH 8.0; 50ml of 10% SDS; and 415ml of ddH₂O. The pH was adjusted to 8.0. The buffer was sterile-filtered, and stored at 4 °C. The pH was re-checked immediately prior to use.

[00369] ChIA-PET Wash Buffer (50ml) contains 500µl of 1M Tris-HCL, pH 8.0 (final 10mM); 100µl of 0.5M EDTA, pH 8.0 (final 1mM); 5ml of 5M NaCl (final 500mM); and 44.4ml of dH₂O.

L. HiChIP

[00370] Alternatively to ChIA-PET, HiChIP was used to analyze chromatin interactions and conformation. HiChIP requires fewer cells than ChIA-PET.

i. Cell crosslinking

[00371] Cells were cross-linked as described in the ChIP protocol above. Crosslinked cells were either stored as pellets at -80°C or used for HiChIP immediately after flash-freezing the cells.

ii. Lysis and restriction

[00372] Fifteen million cross-linked cells were resuspended in 500µL of ice-cold Hi-C Lysis Buffer and rotated at 4°C for 30 minutes. For cell amounts greater than 15 million, the pellet was split in half for contact generation and then recombined for sonication. Cells were spun down at 2500g for 5 minutes, and the supernatant was discarded. The pelleted nuclei were washed once with 500µL of ice-cold Hi-C Lysis Buffer. The supernatant was removed, and the pellet was resuspended in 100µL of 0.5% SDS. The resuspension was incubated at 62°C for 10 minutes, and then 285µL of H₂O and 50µL of 10% Triton X-100 were added to quench the SDS. The resuspension was mixed well, and incubated at 37°C for 15 minutes. Fifty µL of 10X NEB Buffer 2 and 375 U of MboI restriction enzyme (NEB, R0147) was added to the mixture to digest chromatin for 2 hours at 37°C with rotation. For lower starting material, less restriction enzyme was used: 15µL was used for 10-15 million cells, 8µL for 5 million cells, and 4µL for 1 million cells. Heat (62°C for 20 minutes) was used to inactivate MboI.

iii. Biotin Incorporation and Proximity Ligation

[00373] To fill in the restriction fragment overhangs and mark the DNA ends with biotin, 52µL of fill-in master mix was reacted by combining 37.5µL of 0.4mM biotin-dATP (Thermo 19524016); 1.5µL of 10mM dCTP, dGTP, and dTTP; and 10µL of 5U/µL DNA Polymerase I, Large (Klenow) Fragment (NEB, M0210). The mixture was incubated at 37°C for 1 hour with rotation.

[00374] 948µL of ligation master mix was added. Ligation Master Mix contained 150µL of 10X NEB T4 DNA ligase buffer with 10mM ATP (NEB, B0202); 125µL of 10% Triton X-100; 3µL of 50mg/mL BSA; 10µL of 400 U/µL T4 DNA Ligase (NEB, M0202); and 660µL of water. The mixture was incubated at room temperature for 4 hours with rotation. The nuclei were pelleted at 2500g for 5 minutes, and the supernatant was removed.

iv. Sonication

[00375] For sonication, the pellet was brought up to 1000µL in Nuclear Lysis Buffer. The sample was transferred to a Covaris millitube, and the DNA was sheared using a Covaris® E220Evolution™ with the manufacturer recommended parameters. Each tube (15 million cells) was sonicated for 4 minutes under the following conditions: Fill Level 5; Duty Cycle 5%; PIP 140; and Cycles/Burst 200.

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v. Preclearing, Immunoprecipitation, IP Bead Capture, and Washes

[00376] The sample was clarified for 15 minutes at 16,100g at 4°C. The sample was split into 2 tubes of about 400µL each and 750µL of ChIP Dilution Buffer was added. For the Smc1a antibody (Bethyl A300-055A), the sample was diluted 1:2 in ChIP Dilution Buffer to achieve an SDS concentration of 0.33%. 60µL of Protein G beads were washed for every 10 million cells in ChIP Dilution Buffer. Amounts of beads (for preclearing and capture) and antibodies were adjusted linearly for different amounts of cell starting material. Protein G beads were resuspended in 50µL of Dilution Buffer per tube (100µL per HiChIP). The sample was rotated at 4°C for 1 hour. The samples were put on a magnet, and the supernatant was transferred into new tubes. 7.5µg of antibody was added for every 10 million cells, and the mixture was incubated at 4°C overnight with rotation. Another 60µL of Protein G beads for every 10 million cells in ChIP Dilution Buffer was added. Protein G beads were resuspended in 50µL of Dilution Buffer (100 µL per HiChIP), added to the sample, and rotated at 4°C for 2 hours. The beads were washed three times each with Low Salt Wash Buffer, High Salt Wash Buffer, and LiCl Wash Buffer. Washing was performed at room temperature on a magnet by adding 500µL of a wash buffer, swishing the beads back and forth twice by moving the sample relative to the magnet, and then removing the supernatant

vi. ChIP DNA Elution

[00377] ChIP sample beads were resuspended in 100µL of fresh DNA Elution Buffer. The sample beads were incubated at RT for 10 minutes with rotation, followed by 3 minutes at 37°C with shaking. ChIP samples were placed on a magnet, and the supernatant was removed to a fresh tube. Another 100µL of DNA Elution Buffer was added to ChIP samples and incubations were repeated. ChIP sample supernatants were removed again and transferred to a new tube. There was about 200µL of ChIP sample. Ten µL of Proteinase K (20mg/ml) was added to each sample and incubated at 55°C for 45 minutes with shaking. The temperature was increased to 67°C, and the samples were incubated for at least 1.5 hours with shaking. The DNA was Zymo-purified (Zymo Research, #D4014) and eluted into 10µL of water. Post-ChIP DNA was quantified to estimate the amount of Tn5 needed to generate libraries at the correct size distribution. This assumed that contact libraries were generated properly, samples were not over sonicated, and that material was robustly captured on streptavidin beads. SMC1 HiChIP with 10 million cells had an expected yield of post-ChIP DNA from 15ng–50ng. For libraries with greater than 150ng of post-ChIP DNA, materials were set aside and a maximum of 150ng was taken into the biotin capture step

vii. Biotin Pull-Down and Preparation for Illumina Sequencing

[00378] To prepare for biotin pull-down, 5µL of Streptavidin C-1 beads were washed with Tween Wash Buffer. The beads were resuspended in 10µL of 2X Biotin Binding Buffer and

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added to the samples. The beads were incubated at RT for 15 minutes with rotation. The beads were separated on a magnet, and the supernatant was discarded. The beads were washed twice by adding 500µL of Tween Wash Buffer and incubated at 55°C for 2 minutes while shaking. The beads were washed in 100µL of 1X (diluted from 2X) TD Buffer. The beads were resuspended in 25µL of 2X TD Buffer, 2.5µL of Tn5 for each 50ng of post-ChIP DNA, and water to a volume of 50µL.

[00379] The Tn5 had a maximum amount of 4 µL. For example, for 25ng of DNA transpose, 1.25µL of Tn5 was added, while for 125ng of DNA transpose, 4µL of Tn5 was used. Using the correct amount of Tn5 resulted in proper size distribution. An over-transposed sample had shorter fragments and exhibited lower alignment rates (when the junction was close to a fragment end). An undertransposed sample has fragments that are too large to cluster properly on an Illumina sequencer. The library was amplified in 5 cycles and had enough complexity to be sequenced deeply and achieve proper size distribution regardless of the level of transposition of the library.

[00380] The beads were incubated at 55°C with interval shaking for 10 minutes. Samples were placed on a magnet, and the supernatant was removed. Fifty mM EDTA was added to samples and incubated at 50°C for 30 minutes. The samples were then quickly placed on a magnet, and the supernatant was removed. The samples were washed twice with 50mM EDTA at 50°C for 3 minutes, then were removed quickly from the magnet. Samples were washed twice in Tween Wash Buffer for 2 minutes at 55°C, then were removed quickly from the magnet. The samples were washed with 10mM Tris-HCl, pH8.0.

viii. PCR and Post-PCR Size Selection

[00381] The beads were resuspended in 50µL of PCR master mix (use Nextera XT DNA library preparation kit from Illumina, #15028212 with dual-Index adapters # 15055289). PCR was performed using the following program. The cycle number was estimated using one of two methods: (1) A first run of 5 cycles (72°C for 5 minutes, 98°C for 1 minute, 98°C for 15 seconds, 63°C for 30 seconds, 72°C for 1 minute) was performed on a regular PCR and then the product was removed from the beads. Then, 0.25X SYBR green was added, and the sample was run on a qPCR. Samples were pulled out at the beginning of exponential amplification; or (2) Reactions were run on a PCR and the cycle number was estimated based on the amount of material from the post-ChIP Qubit (greater than 50ng was run in 5 cycles, while approximately 50ng was run in 6 cycles, 25ng was run in 7 cycles, 12.5ng was run in 8 cycles, etc.).

[00382] Libraries were placed on a magnet and eluted into new tubes. The libraries were purified using a kit from Zymo Research and eluted into 10µL of water. A two-sided size selection was performed with AMPure XP beads. After PCR, the libraries were placed on a magnet and eluted into new tubes. Then, 25µL of AMPure XP beads were added, and the supernatant was kept to capture fragments less than 700 bp. The supernatant was transferred to a

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new tube, and 15µL of fresh beads were added to capture fragments greater than 300 bp. A final elution was performed from the Ampure XP beads into 10µL of water. The library quality was verified using a Bioanalyzer.

ix. Buffers

[00383] Hi-C Lysis Buffer (10mL) contained 100µL of 1M Tris-HCl pH 8.0; 20µL of 5M NaCl; 200µL of 10% NP-40; 200µL of 50X protease inhibitors; and 9.68mL of water. Nuclear Lysis Buffer (10mL) contained 500µL of 1M Tris-HCl pH 7.5; 200µL of 0.5M EDTA; 1mL of 10% SDS; 200µL of 50X Protease Inhibitor; and 8.3mL of water. CHIP Dilution Buffer (10mL) contained 10µL of 10% SDS; 1.1mL of 10% Triton X-100; 24µL of 500mM EDTA; 167µL of 1M Tris pH 7.5; 334µL of 5M NaCl; and 8.365mL of water. Low Salt Wash Buffer (10mL) contained 100µL of 10% SDS; 1mL of 10% Triton X-100; 40µL of 0.5M EDTA; 200µL of 1M Tris-HCl pH 7.5; 300µL of 5M NaCl; and 8.36mL of water. High Salt Wash Buffer (10mL) contained 100µL of 10% SDS; 1mL of 10% Triton X-100; 40µL of 0.5M EDTA; 200µL of 1M Tris-HCl pH 7.5; 1mL of 5M NaCl; and 7.66mL of water. LiCl Wash Buffer (10mL) contained 100µL of 1M Tris pH 7.5; 500µL of 5M LiCl; 1mL of 10% NP-40; 1mL of 10% Na-deoxycholate; 20µL of 0.5M EDTA; and 7.38mL of water.

[00384] DNA Elution Buffer (5mL) contained 250µL of fresh 1M NaHCO₃; 500µL of 10% SDS; and 4.25mL of water. Tween Wash Buffer (50mL) contained 250µL of 1M Tris-HCl pH 7.5; 50µL of 0.5M EDTA; 10mL of 5M NaCl; 250µL of 10% Tween-20; and 39.45mL of water. 2X Biotin Binding Buffer (10mL) contained 100µL 1M Tris-HCl pH 7.5; 20µL of 0.5M; 4mL of 5M NaCl; and 5.88mL of water. 2X TD Buffer (1mL) contains 20µL of 1M Tris-HCl pH 7.5; 10µL of 1M MgCl₂; 200µL of 100% Dimethylformamide; and 770µL of water.

M. Drug dilutions for administration to hepatocytes

[00385] Prior to compound treatment of hepatocytes, 100mM stock drugs in DMSO were diluted to 10mM by mixing 0.1mM of the stock drug in DMSO with 0.9ml of DMSO to a final volume of 1.0ml. Five µl of the diluted drug was added to each well, and 0.5ml of media was added per well of drug. Each drug was analyzed in triplicate. Dilution to 1000x was performed by adding 5µl of drug into 45µl of media, and the 50µl being added to 450µl of media on cells.

[00386] Bioactive compounds were also administered to hepatocytes. To obtain 1000x stock of the bioactive compounds in 1ml DMSO, 0.1 ml of 10,000X stock was combined with 0.9ml DMSO.

Example 2. RNA-seq study for stimulated hepatocytes

[00387] To identify small molecules that modulate urea cycle enzymes, primary human hepatocytes were prepared as a monoculture, and at least one small molecule compound was applied to the cells.

[00388] RNA-seq was performed to determine the effects of the compounds on the expression of urea cycle enzymes in hepatocytes. Fold change was calculated by dividing the level of expression in the cell system that had been perturbed by the level of expression in an unperturbed system. Changes in expression having a p-value ≤ 0.05 were considered significant.

[00389] Compounds used to perturb the signaling centers of hepatocytes include at least one compound listed in Table 1. In the table, compounds are listed with their ID, target, pathway, and pharmaceutical action. Most compounds chosen as perturbation signals are known in the art to modulate at least one canonical cellular pathway. Some compounds were selected from compounds that failed in Phase III clinical evaluation due to lack of efficacy.

Table 1. Compounds used in RNA-seq

ID	Compound Name	Target	Pathway	Action
1	Simvastatin	HMG-CoA reductase	Metabolic	Inhibitor
2	Adapin (doxepin)	H ₁ histamine, α -adrenoreceptors	Histamine receptor signaling	Antagonist
4	Danazol	ER, AR, Progesteron receptor	Estrogen signaling	Agonist
5	Nefazodone	HTR2A	Calcium signaling	Antagonist
6	Rosiglitazone maleate	PPAR γ	PPAR signaling	Agonist
7	Sulpiride	D ₂ dopamine	cAMP signaling	Antagonist
8	Captopril	MMP2	Estrogen signaling	Inhibitor
9	atenolol	ADRB1	Adrenergic signaling	Antagonist
10	Ranitidine	H ₂ histamine receptor	Histamine receptor signaling	Antagonist
11	Metformin	AMPK	Insulin & AMPK signaling	Activator
12	imatinib	RTK, Bcr-Abl	PDGFR, ABL signaling	Inhibitor
13	Papaverine	phosphodiesterase	AMPK signaling	Inhibitor
14	Amiodarone	Adrenergic receptor β , CYP	Adrenergic signaling	Antagonist
15	Nitrofurantoin	pyruvate-flavodoxin oxidoreductase	antibiotic	Activator
16	prednisone	GR	GR signaling	Agonist
17	Penicillamine(D-)	copper	copper chelation	Chelator
18	Disopyramide	SCN5A	Adrenergic signaling	Inhibitor
19	Rifampicin	PXR	PXR	Inhibitor
20	Benzbromarone	xanthine oxidase, CYP2C9	uric acid formation	Inhibitor
21	isoniazid	CYP2C19, CYP3A4	unknown	Inhibitor
22	Acetaminophen (paracetamol)	COX1/2	COX	Inhibitor
23	Ritonavir	CYP3A4, Pol polyprotein	HIV Transcription	Inhibitor
24	SGI-1776	PIM	JAK/STAT signaling	Inhibitor
25	Valproate	HDAC9, glucuronyl transferase, epoxide hydrolase	unknown	Inhibitor
26	Ibuprofen	COX, PTGS2	COX	Inhibitor
27	Propylthiouracil	thyroperoxidase	Thyroid hormone synthesis	Inhibitor
28	rapamycin	mTOR	mTOR signaling	Inhibitor
29	BIO	GSK-3	WNT, TGF beta signaling	Inhibitor
30	ATRA	RXR β , RXR γ , RAR γ	RAR signaling	Agonist
31	Xav939	tankyrase	WNT & PARP pathway	Inhibitor
32	bms189453	RARB	Nuclear Receptor transcription	Agonist
33	dorsomorphin	ALK	TGF beta signaling	Inhibitor
34	BMP2	BMPRIIA	TGF beta signaling	Agonist
35	BMS777607	Met	Ras signaling	Inhibitor
36	bms833923	SMO	Hedgehog signaling	Antagonist
37	dmPGE2	EPR, PGDH	EP receptor signaling	Agonist
38	MK-0752	γ -secretase	NOTCH signaling	Inhibitor
39	N-Acetylpuromycin	SnoN, SKI, SKIL	TGF beta signaling	Modulator
40	LY 364947	TGF- β RI, TGFR-I, T β R-I, ALK-5	TGF beta signaling	Inhibitor

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41	Enzastaurin	PKC	Epigenetics; TGF-beta/Smad	Inhibitor
42	DMXAA	Unclear	Tumor necrosis	Inhibitor
43	BSI-201	PARP	Cell Cycle/DNA Damage; Epigenetics	Inhibitor
44	Darapladib	Phospholipase	Others	Inhibitor
45	Selumetinib	MEK	MAPK/ERK Pathway	Inhibitor
46	Peramivir (trihydrate)	Influenza Virus	Anti-infection	Inhibitor
47	Palifosfamide	DNA alkylator/crosslinker	Cell Cycle/DNA Damage	
48	Evacetrapib	CETP	Others	Inhibitor
49	Cediranib	VEGFR	Protein Tyrosine Kinase/RTK	Inhibitor
50	R788 (fostamatinib, disodium hexahydrate)	Syk	Protein Tyrosine Kinase/RTK	Inhibitor
51	Torcetrapib	CETP	Others	Inhibitor
52	Tivozanib	VEGFR	Protein Tyrosine Kinase/RTK	Inhibitor
53	17-AAG (Tanespimycin)	HSP	Cell Cycle/DNA Damage Metabolic Enzyme/Protease	Inhibitor
54	Zibotentan	Endothelin Receptor	GPCR/G protein	Antagonist
55	Semagacestat	γ -secretase	Neuronal Signaling Stem Cells/Wnt	Inhibitor
56	Dalcetrapib	CETP	Others	Inhibitor
57	Latrepirdine (dihydrochloride)	AMPK	Epigenetics; PI3K/Akt/mTOR	Activator
58	CMX001 (Brincidofovir)	CMV	Anti-infection	NA
59	Vicriviroc (maleate)	CCR	GPCR/G protein; Immunology/Inflammation	Antagonist
60	Temsirolimus	mTOR	PI3K/Akt/mTOR	Inhibitor
61	Preladenant	Adenosine Receptor	GPCR/G protein	Antagonist
62	EVP-6124 (hydrochloride) (encenicline)	nAChR	Membrane Transporter/Ion Channel	Activator
63	Bitopertin	GlyT1	Membrane Transporter/Ion Channel	Inhibitor
64	Latrepirdine	AMPK	Epigenetics; PI3K/Akt/mTOR	Inhibitor
65	Vanoxerine (dihydrochloride)	Dopamine Reuptake Inhibitor	Neuronal Signaling	Inhibitor
66	CO-1686 (Rociletinib)	EGFR	JAK/STAT Signaling Protein Tyrosine Kinase/RTK	Inhibitor
67	Laropiprant (tredaptive)	Prostaglandin Receptor	GPCR/G protein	Antagonist
68	Bardoxolone	Keap1-Nrf2	NF- κ B	Activator
69	VX-661 (tezacaptor)	CFTR	Membrane transporter/ion channel	Corrector
70	INNO-206 (aldoxonibicin)	Topoisomerase	Cell Cycle/DNA Damage	NA
71	LY404039 (pomaglumetad methionil (mGlu2/3))	mGluR	GPCR/G protein	Inhibitor
72	Perifosine (KRX- 0401)	AKT	PI3K/AKT	Inhibitor
73	Cabozantinib (XL184, BMS-907351)	VEGFR2, MET, Ret, Kit, Flt- 1/3/4, Tie2, and AXL	MET	Inhibitor
74	Dacomitinib (PF299804, PF299)	EGFR, ErbB2, ErbB4	AKT/ERK, HER	Inhibitor
75	Pacritinib (SB1518)	FLT3, JAK2, TYK2, JAK3, JAK1	JAK-STAT	Inhibitor
76	TH-302 (Evofofosfamide)	hypoxic regions	Unclear	NA
77	α -PHP	Unclear	Unclear	NA
78	LY 2140023 (Pomaglumetad methionil-LY404039)	mGlu ₂ & mGlu ₃	Gai/o protein-dependent	Activator

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79	TP-434 (Eravacycline)	Antibiotic resistance mechanisms	Tetracycline-specific efflux	Inhibitor
80	TC-5214 (S-+)-MecaMylaMine Hydrochloride)	Nicotinic acetylcholine receptors	Base excision repair and homologous recombination repair	Antagonist
81	Rolofylline (KW-3902)	A1 adenosine receptor	Unclear	Antagonist
82	Amigal (Deoxygalactonojirimycin hydrochloride)	a-galactosidase	Stress signaling	Inhibitor
83	NOV-002 (oxidized L-Glutathione)	gamma-glutamyl-transpeptidase (GGT)	Glutathione pathway	NA
84	bms-986094 (inx-189)	NS5B	Unclear	Inhibitor
85	TC-5214 (R-Mecamylamine hydrochloride)	Nicotinic receptors	Base excision repair and homologous recombination repair	Antagonist
86	Ganaxolone	GBAA receptors	Unclear	Modulator
87	Irinotecan Hydrochloride Trihydrate	DNA Topo I	Unclear	Inhibitor
88	TFP	D2R, Calmodulin	Calmodulin	Inhibitor
89	Perphenazine	D2R, Calmodulin	Calmodulin	Inhibitor
90	A3-HCl	CKI, CKII, PKC, PKA	WNT, Hedgehog, PKC, PKA	Inhibitor
91	FICZ	Aryl hydrocarbon receptor	Aryl hydrocarbon receptor	Agonist
92	Pifithrin-a	p53	p53	Inhibitor
93	Deferoxamine mesylate	HIF	Hypoxia activated	Inhibitor
94	Insulin	InsR	IGF-1R/InsR	Activator
95	Phorbol 12,13-dibutyrate	PKC	PKC	Activator
96	RU 28318	MR	Mineralcorticoid receptor	Antagonist
97	Bryostatins	PKC	PKC	Activator
98	DY 268	FXR	FXR	Antagonist
99	GW 7647	PPAR α	PPAR	Agonist
100	CI-4AS-1	AR	Androgen receptor	Agonist
101	T0901317	LXR	LXR	Agonist
102	BMP2	BMPRI A	TGF-B	Activator
103	22S-Hydroxycholesterol	LXR	LXR	Inhibitor
104	CALPI	Calmodulin	Calmodulin	Activator
105	CALP3	Calmodulin	Calmodulin	Activator
106	Forskolin	Adenylyl cyclase	cAMP related	Activator
107	Dexamethasone	GR	Glucocorticoid receptor	Activator
108	IFN- γ	IFNGR1/IFNGR2	JAK/STAT	Activator
109	TGF-b	TGF-beta Receptor	TGF-B	Activator
110	TNF- α	TNF-R1/TNF-R2	NF-kB, MAPK, Apoptosis	Activator
111	PDGF	Pan-PDGFR	PDGFR	Activator
112	IGF-1	IGF-1R	IGF-1R/InsR	Activator
113	FGF	FGFR	FGFR	Activator
114	EGF	Pan-ErbB	EGFR	Activator
115	HGF/SF	c-Met	c-MET	Activator
116	TCS 359	FLT3	Protein Tyrosine Kinase/RTK	Inhibitor
117	Cobalt chloride	HIF1	Hypoxia activated	Inducer
118	CH223191	AhR	Aryl hydrocarbon receptor	Antagonist
119	Echinomycin	HIF	Hypoxia activated	Inhibitor
120	PAF C-16	MEK	MAPK	Activator
121	Bexarotene	RXR	RXR	Agonist
122	CD 2665	RAR	RAR	Antagonist
123	Pifithrin- μ	p53	p53	Inhibitor
124	EB1089	VDR	Vitamin D Receptor	Agonist
125	BMP4	TGF-beta	TGF-B	Activator

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126	IWP-2	Wnt	WNT	Inhibitor
127	RITA (NSC 652287)	p53	p53	Inhibitor
128	Calcitriol	VDR	Vitamin D Receptor	Agonist
129	ACEA	CB1	Cannabinoid receptor	Agonist
130	Rimonabant	CB1	Cannabinoid receptor	Antagonist
131	Otenabant	CB1	Cannabinoid receptor	Antagonist
132	DLPC	LRH-1/NR5A2	LHR-1	Agonist
133	LRH-1 antagonist	LRH-1/NR5A3	LHR-1	Antagonist
134	Wnt3a	FRIZZLED	WNT	Activator
135	Activin	TGF-beta	TGF-B	Activator
136	Nodal	TGF-beta	TGF-B	Activator
137	Anti mullerian hormone	TGF-bcta	TGF-B	Activator
138	GDF2 (BMP9)	TGF-beta	TGF-B	Activator
139	GDF10 (BMP3b)	TGF-beta	TGF-B	Activator
140	Oxoglucine	PI3K/Akt	PI3K/AKT	Activator
141	BMS 195614	RAR	RAR	Antagonist
142	LDN193189	ALK2/3	TGF-B	Inhibitor
143	Varenicline Tartrate	AchR	Acetylcholine receptor	Agonist
144	Histamine	Histamine receptor	Histamine receptor	Activator
145	Chloroquine phosphate	ATM/ATR	ATM/ATR	Activator
146	LJI308	RSK1/2/3	S6K	Inhibitor
147	GSK621	AMPK	AMPK	Activator
148	STA-21	STAT3	JAK/STAT	Inhibitor
149	SMI-4a	Pim1	PIM	Inhibitor
150	AMG 337	c-Met	c-MET	Inhibitor
151	Wnt agonist 1	Wnt	WNT	Activator
152	PRI-724	Wnt	WNT	Inhibitor
153	ABT-263	Pan-Bcl-2	BCL2	Inhibitor
154	Axitinib	Pan-VEGFR	VEGFR	Inhibitor
155	Afatinib	Pan-ErbB	EGFR	Inhibitor
156	Bosutinib	Src	Src	Inhibitor
157	Dasatinib	Bcr-Abl	ABL	Inhibitor
158	Masitinib	c-Kit	c-KIT	Inhibitor
159	Crizotinib	c-Met	c-MET	Inhibitor
160	PHA-665752	c-Met	c-MET	Inhibitor
161	GSK1904529A	IGF-1R/InsR	IGF-1R/InsR	Inhibitor
162	GDC-0879	Raf	MAPK	Inhibitor
163	LY294002	Pan-PI3K	PI3K/AKT	Inhibitor
164	OSU-03012	PDK-1	PDK-1	Inhibitor
165	JNJ-38877605	c-Met	c-MET	Inhibitor
166	BMS-754807	IGF-1R/InsR	IGF-1R/InsR	Inhibitor
167	TGX-221	p110b	PI3K/AKT	Inhibitor
168	Regorafenib	Pan-VEGFR	VEGFR	Inhibitor
169	Thalidomide	AR	NF-kB	Antagonist
170	Amuvatinib	PDGFRA	PDGFR	Inhibitor
171	Etomidate	GABA	GABAergic receptor	Inhibitor
172	Glimepiride	Potassium channel	Potassium channel	Inhibitor
173	Omeprazole	Proton pump	Proton pump	Agonist
174	Tipifarnib	Ras	RAS	Inhibitor
175	SP600125	Jnk	MAPK	Inhibitor
176	Quizartinib	FLT3	FLT3	Inhibitor
177	CP-673451	Pan-PDGFR	PDGFR	Inhibitor
178	Pomalidomide	TNF- α	NF-kB	Inhibitor
179	KU-60019	ATM kinase	DNA Damage	Inhibitor
180	BIRB 796	p38	MAPK	Inhibitor
181	RO4929097	Gamma-secretase	NOTCH	Inhibitor
182	Hydrocortisone	GR	Glucocorticoid receptor	Agonist
183	AICAR	AMPK	AMPK	Activator
184	Amlodipine Besylate	Calcium channel	Calcium channel	Inhibitor

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185	DPH	Bcr-Abl	ABL	Activator
186	Taladegib	Hedgehog/Smoothened	Hedgehog/Smoothened	Inhibitor
187	AZD1480	JAK2	JAK/STAT	Inhibitor
188	AST-1306	Pan-ErbB	EGFR	Inhibitor
189	AZD8931	Pan-ErbB	EGFR	Inhibitor
190	Momelotinib	Pan-Jak	JAK/STAT	Inhibitor
191	Cryptotanshinone	STAT3	JAK/STAT	Inhibitor
192	Bethanechol chloride	AchR	Acetylcholine receptor	Activator
193	Clozapine	5-HT	5-HT	Antagonist
194	Dopamine	Dopamine	Dopamine receptor	Agonist
195	Phenformin	AMPK	AMPK	Activator
196	Mifepristone	GR	Glucocorticoid receptor	Antagonist
197	GW3965	LXR	LXR	Agonist
198	WYE-125132 (WYE-132)	mTOR	mTOR	Inhibitor
199	Crenolanib	Pan-PDGFR	PDGFR	Inhibitor
200	PF-04691502	Pan-Akt	PI3K/AKT	Inhibitor
201	GW4064	FXR	FXR	Agonist
202	Sotrastaurin	PKC	PKC	Inhibitor
203	Ipatasertib	Pan-Akt	PI3K/AKT	Inhibitor
204	ARN-509	AR	Androgen receptor	Inhibitor
205	T0070907	PPAR γ	PPAR	Antagonist
206	GO6983	PKC	PKC	Inhibitor
207	Epinephrine	Adrenergic	Adrenergic receptor	Agonist
208	Eletriptan	5-HT	5-HT	Agonist
209	Trifluoperazine	Dopamine	Dopamine receptor	Inhibitor
210	Fexofenadine	Histamine	Histamine receptor	Inhibitor
211	Deoxycorticosterone	MR	Mineralcorticoid receptor	Agonist
212	Tamibarotene	RAR	RAR	Agonist
213	Leucine	mTOR	mTOR	Activator
214	Glycopyrrolate	AchR	Acetylcholine receptor	Antagonist
215	Tiagabine	GABA	GABAergic receptor	Inhibitor
216	Fluoxymesterone	AR	Androgen receptor	Agonist
217	Tamsulosin hydrochloride	Adrenergic	Adrenergic receptor	Antagonist
218	Ceritinib	ALK	ALK	Inhibitor
219	GSK2334470	PDK-1	PDK-1	Inhibitor
220	AZD1208	Pan-PIM	PIM	Inhibitor
221	CGK733	ATM/ATR	DNA Damage	Inhibitor
222	LDN-212854	Pan-TGFB	TGF-B	Inhibitor
223	GZD824 Dimesylate	Bcr-Abl	ABL	Inhibitor
224	AZD2858	Pan-GSK-3	GSK-3	Inhibitor
225	FRAX597	PAK	PAK	Inhibitor
226	SC75741	NF-kB	NF-kB	Inhibitor
227	SH-4-54	Pan-STAT	JAK/STAT	Inhibitor
228	HS-173	p110a	PI3K/AKT	Inhibitor
229	K02288	Pan-TGFB	TGF-B	Inhibitor
230	EW-7197	Pan-TGFB	TGF-B	Inhibitor
231	Decernotinib	Pan-Jak	JAK/STAT	Inhibitor
232	MI-773	p53	p53	Inhibitor
233	PND-1186	FAK	FAK	Activator
234	Kartogenin	SMAD4/5	TGF-B	Activator
235	Picropodophyllin	IGF-1R	IGF-1R/InsR	Inhibitor
236	AZD6738	ATR	ATM/ATR	Inhibitor
237	Smoothened Agonist	Hedgehog/Smoothened	Hedgehog/Smoothened	Agonist
238	Erlotinib	EGFR/ErbB1	EGFR	Inhibitor
239	MHY1485	mTOR	mTOR	Activator
240	SC79	Pan-Akt	PI3K/AKT	Activator
241	meBIO	AhR	Aryl hydrocarbon receptor	Agonist
242	Huperzine	AchE	Acetylcholine receptor	Inhibitor
243	BGJ398	Pan-FGFR	FGFR	Inhibitor

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244	Netarsudil	ROCK	ROCK	Inhibitor
245	Acetylcholine	AchR	Acetylcholine receptor	Agonist
246	Purmorphamine	Hedgehog/Smoothed	Hedgehog/Smoothed	Agonist
247	LY2584702	p70 S6K	S6K	Inhibitor
248	Dorsomorphin	AMPK	AMPK	Inhibitor
249	Glasdegib (PF-04449913)	Hedgehog/Smoothed	Hedgehog/Smoothed	Inhibitor
250	LDN193189	Pan-TGFB	TGF-B	Inhibitor
251	Oligomycin A	ATPase	ATP channel	Inhibitor
252	BAY 87-2243	HIF1	Hypoxia activated	Inhibitor
253	SIS3	SMAD3	TGF-B	Inhibitor
254	BDA-366	Bcl-2	BCL2	Antagonist
255	XMU-MP-1	MST1/2	Hippo	Inhibitor
256	Semaxanib	Pan-VEGFR	VEGFR	Inhibitor
257	BAM7	Bcl-2	BCL2	Activator
258	GDC-0994	Erk	MAPK	Inhibitor
259	SKL2001	Wnt	WNT	Agonist
260	Merestinib	c-Met	c-MET	Inhibitor
261	APS-2-79	MEK	MAPK	Antagonist
262	NSC228155	Pan-ErbB	EGFR	Activator
263	740 Y-P	Pan-PI3K	PI3K/AKT	Activator
264	b-Estradiol	ER	ER	Activator
265	Glucose	GLUTs	metabolic/glycolysis	Activator
266	Transferrin	Transferrin Receptor	Iron transport	Activator
267	AM 580	RAR	RAR	Activator

Example 3. Identification of compounds that modulate expression of urea cycle enzymes

[00390] Analysis of RNA-seq data revealed a number of compounds that caused significant changes in the expression of CPS1, OTC, ASS1, ASL, and/or NAGS. Significance was defined as an FPKM ≥ 1 , a log₂ (fold change) ≥ 0.5 , and a q-value of ≤ 0.05 for selected gene target. RNA-seq results for compounds that significantly modulated at least one target gene are shown in Tables 2-10. Table 2 provides the log₂ fold change for compounds that were observed to significantly increase the expression of CPS1, which is associated with CPS deficiency.

Table 2. CPS1 expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
157	Dasatinib	6.44
50	R788 (fostamatinib disodium hexahydrate)	5.93
156	Bosutinib	5.06
207	Epinephrine	4.85
225	FRAX597	4.61
260	Merestinib	3.96
211	Deoxycorticosterone	3.79
53	17-AAG (Tanespimycin)	3.73

138	GDF2 (BMP9)	3.46
223	GZD824 Dimesylate	3.39
233	PND-1186	2.85
134	Wnt3a	2.6
136	Nodal	2.44
137	Anti mullerian hormone	2.35
110	TNF- α	2.31
135	Activin	2.23
112	IGF-1	2.22
16	prednisone	2.17
111	PDGF	2.12
115	HGF/SF	2.02
114	EGF	1.93
252	BAY 87-2243	1.81
177	CP-673451	1.77
113	FGF	1.72
139	GDF10 (BMP3b)	1.66
250	LDN193189	1.56
170	Amuvatinib	1.46
190	Momelotinib	1.46
119	Echinomycin	1.45
75	Pacritinib (SB1518)	1.33
102	BMP2	1.31
159	Crizotinib	1.08
222	LDN-212854	1.08
169	Thalidomide	1.02
66	CO-1686 (Rociletinib)	0.89
54	Zibotentan	0.81

[00391] Table 3 provides the log₂ fold change for compounds that were observed to significantly increase the expression of OTC, which is associated with OTC deficiency.

Table 3. OTC expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
177	CP-673451	2.63

75	Pacritinib (SB1518)	2.33
119	Echinomycin	2.08
199	Crenolanib	1.23
169	Thalidomide	1.21
170	Amuvatinib	1.15
157	Dasatinib	1.06
190	Momelotinib	0.95
135	Activin	0.94
134	Wnt3a	0.92
70	INNO-206 (aldoxorubicin)	0.87
110	TNF- α	0.87
137	Anti mullerian hormone	0.81
123	Pifithrin- μ	0.79
111	PDGF	0.77
112	IGF-1	0.76
225	FRAX597	0.69
136	Nodal	0.68
114	EGF	0.64
113	FGF	0.56
115	HGF/SF	0.56
180	BIRB 796	0.56

[00392] Table 3A provides additional data for compounds that were observed to increase the expression of OTC, which is associated with OTC deficiency. Compounds assayed at 10uM final concentration, except for compounds HSP-990 and Retaspimycin Hydrochloride which were assayed at 1uM final concentration.

Table 3A. OTC expression modulated by compounds

ID	Name/Company	MoA	Max Value (OTC/Geo Mean of HKs; DMSO=1)
248	Dorsomorphin	AMPK and BMPR1	4.78
600	Foretinib (GSK1363089)	c-Met, VEGFR	4.09
327	CCCp	TBK1 /IRF3	4.06
190	Momelotinib	JAK	3.52

630	PF-00562271	FAK	3.3
626	Mubritinib (TAK 165)	HER2	3.23
316	XL228	BCR-Abl, IGF-1R	2.92
551	Sunitinib	PDGFR	2.62
180	BIRB 796	p38 MAPK & JNK2	2.61
326	BX795	TBK1	2.57
70	Aldoxorubicin	Doxorubicin prodrug	2.36
673	BMS-214662	Farnesyl Transferase Inhibitor	2.27
654	Lifirafenib (BGB-283)	Raf,EGFR	2.26
685	Pamapimod	p38 MAPK	1.73
323	PF-04929113 (SNX- 5422)	HSP90 & HER2	1.73
53	17-AAG	HSP90 & HER2	1.69
320	BIB021	HSP90	1.66
277	Baricitinib	JAK 1 & 2	1.66
170	Amuvatinib	PDGFA	3.21
199	Crenolanib	Pan-PDGFR	2.79
497	Pazopanib	c-Kit,PDGFR,VEGFR	1.48
322	HSP-990	HSP90	1.5 (compound tested at 1uM)
925	Retaspimycin Hydrochloride	HSP90 ATPase	1.48 (compound tested at 1uM)
187	AZD1480	JAK2	1.46
325	MRT67307	TBK1	1.68
68	Bardoxolone	NF-κB	2.76
177	CP-673451	Pan-PDGFR	3.97
313	Tofacitinib citrate	JAK1 & 2	1.6
311	LY2784544	JAK2	2.78
684	R1487 Hydrochloride	p38 MAPK	1.64
52	Tivozanib	VEGFR 1/2/3	1.57
268	Regorafenib	Pan-VEGFR	1.54
300	GLPG0634	Pan-JAK	1.54
632	PH-797804	p38 MAPK	1.53
55	Semagacestat	β-amyloid & Notch	1.5
71	Pomaglometad	mGlu2/mGlu3 (agonist	1.49
253	SIS3	SMAD3 (TGFB)	1.46
587	Linifanib (ABT-869)	CSF-1R,PDGFR,VEGFR	2.87
609	Regorafenib (BAY 73- 4506)	VEGFR, PDGFRβ	2.07
631	TAK-901	Aurora Kinase	2.9
919	AS602801 (Bentamapimod)	JNK	2.75
603	YM155 (Sapantronium Bromide)	Survivin	3.16

Example 4. Use of siRNA agents to up-regulate OTC expression

[00393] Primary human hepatocytes were reversed transfected with 6pmol siRNA using RNAiMAX Reagent (ThermoFisher Cat No13778030) in 24 well format, 1ul per well (for a final conc of 10nM). The following morning, the medium was removed and replaced with Modified Maintenance Medium (see above) for an additional 24 hrs. The entire treatment lasted 48hrs, at which point the medium was removed and replaced with RLT Buffer for RNA extraction (Qiagen RNeasy 96 QIAcube HT Kit Cat#74171). siRNAs were obtained from Dharmacon and are a pool of four siRNA duplex all designed to target distinct sites within the specific gene of interest (known as “SMARTpool”). The siRNA catalog numbers are listed in Table 3B, below. They were tested against the control non-targeting siRNA D-001206-13-05.

[00394] Isolated RNA was processed for cDNA synthesis and qPCR as described above.

Taqman assay used for OTC measurement: Hs00166892_m1

Table 3B. siRNA Agents up-regulate OTC

siRNA	Max fold change in OTC	Catalog Number
JAK1	3.51	M-003145-02-0005
WWTR1	3.43	M-016083-00-0005
YAP1	2.93	M-046247-01- 0005
CSF1R	2.80	D-003109-06
LYN	2.51	D-003153-03
SMAD3	2.49	M-020067-00-0005
NTRK1	2.25	D-003159-05
EPHB3	2.23	D-003123-09
EPHB4	2.21	D-003124-05
FGFR4	2.16	D-003134-05
INSR	2.13	D-003014-05
KDR	2.12	D-003148-05
FLT1	2.09	D-003136-05
FGFR2	2.07	D-003132-05
EPHB2	1.91	D-003122-07
PDGFRB	1.90	M-003163-03-0005
IRF5	1.90	M-011706-00- 0005
FGFR1	1.87	D-003131-09
EPHB1	1.85	D-003121-05
FYN	1.81	D-003140-09
FLT4	1.71	D-003138-05
Yy1	1.71	M-011796-02-0005
IRF1	1.70	M-011704-01- 0005
IGF-1	1.69	D-003012-06

SMAD1	1.65	M-012723-01-0005
DDR1	1.64	D-003111-08
HSP90AA1	1.52	M-005186-02-0005
SMAD2	1.44	M-003561-01-0005

[00395] Table 4 provides the log2 fold change for compounds that were observed to significantly increase the expression of ASS1, which is associated with ASS1 deficiency.

Table 4. ASS1 expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
157	Dasatinib	1.86
177	CP-673451	1.52
119	Echinomycin	1.44
138	GDF2 (BMP9)	1.34
75	Pacritinib (SB1518)	1.27
207	Epinephrine	1.08
225	FRAX597	1.06
156	Bosutinib	0.86
79	TP-434 (Eravacycline)	0.76
102	BMP2	0.75
149	SMI-4a	0.69
170	Amuvatinib	0.69
199	Crenolanib	0.64
211	Deoxycorticosterone	0.6
70	INNO-206 (aldoxorubicin)	0.58
110	TNF- α	0.58
101	T0901317	0.55

[00396] Table 5 provides the log2 fold change for compounds that were observed to significantly increase the expression of ASL, which is associated with ASL deficiency.

Table 5. ASL expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
177	CP-673451	1.81
119	Echinomycin	1.76

75	Pacritinib (SB1518)	1.45
157	Dasatinib	0.94
251	Oligomycin A	0.84
260	Merestinib	0.84
170	Amuvatinib	0.81
199	Crenolanib	0.76
207	Epinephrine	0.6
252	BAY 87-2243	0.58
169	Thalidomide	0.56

[00397] Table 6 provides the log2 fold change for compounds that were observed to significantly increase the expression of NAGS, which is associated with NAGS deficiency.

Table 6. NAGS expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
224	AZD2858	2.16
41	Enzastaurin	2.03
156	Bosutinib	2.02
256	Semaxanib	2
70	INNO-206 (aldoxorubicin)	1.63
79	TP-434 (Eravacycline)	1.5
195	Phenformin	1.38
159	Crizotinib	1.35
149	SMI-4a	1.25
157	Dasatinib	1.21
128	Calcitriol	1.2
123	Pifithrin- μ	1.18
160	PHA-665752	1.15
44	Darapladib	1.13
169	Thalidomide	1.1
66	CO-1686 (Rociletinib)	1.05
164	OSU-03012	1.03
16	prednisone	1
219	GSK2334470	1

155	Afatinib	0.98
52	Tivozanib	0.95
259	SKL2001	0.95
162	GDC-0879	0.85
62	EVP-6124 (hydrochloride) (encenicline)	0.75
184	Amlodipine Besylate	0.73
101	T0901317	0.72
206	GO6983	0.7
135	Activin	0.69
198	WYE-125132 (WYE-132)	0.64
253	SIS3	0.64
138	GDF2 (BMP9)	0.63
95	Phorbol 12, 13-dibutyrate	0.62
122	CD 2665	0.62
238	Erlotinib	0.62
218	Ceritinib	0.61
102	BMP2	0.6
88	TFP	0.58
115	HGF/SF	0.56
100	CI-4AS-1	0.55

[00398] Table 7 provides the log₂ fold change for compounds that were observed to significantly increase the expression of ARG1, which is associated with Arginase deficiency.

Table 7. ARG1 expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
50	R788 (fostamatinib disodium hexahydrate)	3.74
157	Dasatinib	3.29
177	CP-673451	3.21
260	Merestinib	2.7
119	Echinomycin	2.54
170	Amuvatinib	2.5
207	Epinephrine	2.28
156	Bosutinib	2.27
134	Wnt3a	2.07
137	Anti mullerian Hormone	2.06
136	Nodal	2.05
135	Activin	2.01
112	IGF-1	1.89

53	17-AAG (Tanespimycin)	1.87
110	TNF-a	1.87
123	Pifithrin-u	1.87
111	PDGF	1.85
75	Pacritinib (SB1518)	1.84
138	GDF2 (BMP9)	1.79
199	Crenolanib	1.7
16	prednisone	1.59
115	HGF/SF	1.51
190	Momelotinib	1.51
114	EGF	1.46
211	Dcoxycorticosterone	1.37
113	FGF	1.24
169	Thalidomide	1.16
195	Phenformin	1.14
52	Tivozanib	1.1
252	BAY 87-2243	1.09
223	GZD824 Dimesylate	1.06
139	GDF10 (BMP3b)	0.97
233	PND-1186	0.93
225	FRAX597	0.82
102	BMP2	0.81
251	Oligomycin A	0.75
19	Rifampicin	0.72
38	MK-0752	0.71

[00399] Table 8 provides the log₂ fold change for compounds that were observed to significantly increase the expression of SLC25A15, which is associated with ORNT1 deficiency.

Table 8. SLC25A15 expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
157	Dasatinib	2.76
225	FRAX597	2.51
260	Merestinib	2.01
50	R788 (fostamatinib disodium hexahydrate)	1.68
156	Bosutinib	1.25
84	bms-986094 (inx-189)	1.18
207	Epinephrine	1.14
138	GDF2 (BMP9)	1.09
119	Echinomycin	0.96
211	Corticosterone	0.96
112	IGF-1	0.8
177	CP-673451	0.76
223	GZD824 Dimesylate	0.73
230	EW-7197	0.72
111	PDGF	0.71
134	Wnt3a	0.7

[00400] Table 9 provides the log₂ fold change for compounds that were observed to significantly increase the expression of SLC25A13, which is associated with Citrin deficiency.

Table 9. SLC25A13 expression modulated by compounds

ID	Compound	Fold change (Log 2) vs untreated
88	TFP	0.57
53	17-AAG (Tanespimycin)	0.71

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[00401] Table 10 provides the list of compounds that were observed to significantly increase expression of multiple urea cycle-related genes.

Table 10. Compounds that modulate multiple genes

ID	Compound	Pathway	Up-regulated Genes
157	Dasatinib	ABL	CPS1, ASS1, ASL, OTC, NAGS, ARG1, SLC25A15
119	Echinomycin	Hypoxia activated	CPS1, ASS1, ASL, OTC, ARG1, SLC25A15
177	CP-673451	PDGFR	CPS1, ASS1, ASL, OTC, ARG1, SLC25A15
138	GDF2 (BMP9)	TGF-B	ASS1, CPS1, NAGS, ARG1, SLC25A15
156	Bosutinib	Src	ASS1, CPS1, NAGS, ARG1, SLC25A15
207	Epinephrine	Adrenergic receptor	CPS1, ASS1, ASL, ARG1, SLC25A15
75	Pacritinib (SB1518)	JAK-STAT	CPS1, ASS1, ASL, OTC, ARG1
170	Amuvatinib	PDGFR	CPS1, ASS1, ASL, OTC, ARG1
225	FRAX597	PAK	CPS1, ASS1, OTC, ARG1, SLC25A15
169	Thalidomide	NF-kB	NAGS, CPS1, ALS, OTC, ARG1
199	Crenolanib	PDGFR	ASS1, ASL, OTC, ARG1
102	BMP2	TGF-B	ASS1, CPS1, NAGS, ARG1
211	Deoxycorticosterone	Mineralcorticoid receptor	CPS1, ASS1, ARG1, SLC25A15
110	TNF- α	NF-kB, MAPK, Apoptosis	CPS1, ASS1, OTC, ARG1
134	Wnt3a	WNT	CPS1, OTC, ARG1, SLC25A15
111	PDGF	PDGFR	CPS1, OTC, ARG1, SLC25A15
112	IGF-1	IGF-1R/InsR	CPS1, OTC, ARG1, SLC25A15
135	Activin	TGF-B	NAGS, CPS1, OTC, ARG1
115	HGF/SF	c-MET	NAGS, CPS1, OTC, ARG1

53	17-AAG (Tanespimycin)	Cell Cycle/DNA Damage; Metabolic Enzyme/Protease	CPS1, ARG1, SLC25A13
50	R788 (fostamatinib disodium hexahydrate)	Protein Tyrosine Kinase/RTK	CPS1, ARG1, SLC25A15
223	GZD824 Dimesylate	ABL	CPS1, ARG1, SLC25A15
252	BAY 87-2243	Hypoxia activated	CPS1, ASL, ARG1
16	prednisone	GR	CPS1, NAGS, ARG1
136	Nodal	TGF-B	CPS1, OTC, ARG1
190	Momelotinib	JAK/STAT	CPS1, OTC, ARG1
113	FGF	FGFR	CPS1, OTC, ARG1
114	EGF	EGFR	CPS1, OTC, ARG1
137	Anti mullerian hormone	TGF-B	CPS1, OTC, ARG1
70	INNO-206 (aldoxorubicin)	Cell Cycle/DNA Damage	NAGS, ASS1, OTC
123	Pifithrin- μ	p53	OTC, NAGS, ARG1

[00402] As shown above, Dasatinib was observed to significantly up-regulate the expression of seven urea cycle genes with log2 fold changes ≥ 1 . Echinomycin and CP-673451 were observed to significantly up-regulate the expression of six urea cycle genes. GDF2 (BMP9), Bosutinib, Epinephrine, Pacritinib (SB1518), Amuvatinib, FRAX597, and Thalidomide were observed to significantly up-regulate the expression of five urea cycle genes. Crenolanib, BMP2, Deoxycorticosterone, TNF- α , Wnt3a, PDGF, IGF-1, Activin, and HGF/SF were observed to significantly up-regulate the expression of four urea cycle genes. 17-AAG (Tanespimycin), R788 (fostamatinib disodium hexahydrate), GZD824 Dimesylate, BAY 87-2243, prednisone, Nodal, Momelotinib, FGF, EGF, Anti mullerian hormone, INNO-206 (aldoxorubicin), and Pifithrin- μ were observed to significantly up-regulate the expression of three urea cycle genes.

[00403] Dasatinib is a novel, potent and multi-targeted inhibitor that targets Abl, Src and c-Kit. This suggest that inhibiting signaling molecules, particularly Abl, Src or c-Kit, in the Abl-, Src- or c-Kit-mediated signaling pathways may potentially up-regulate enzymes of the urea cycle.

[00404] Mubritinib (TAK-165) is a potent inhibitor of HER2/ErbB2 with IC₅₀ of 6 nM in BT-474 cells.

[00405] XL228 is designed to inhibit the insulin-like growth factor type-1 receptor (IGF1R), Src and Abl tyrosine kinases – targets that play crucial roles in cancer cell proliferation, survival and metastasis.

[00406] Three identified compounds, Foretinib/XL880, Regorafenib, are known modulators of the Vascular endothelial growth factor receptor (VEGFR) family mediated signaling pathway. This suggest that modulating signaling molecules, particularly VEGFRs, in the VEGFR-mediated signaling pathway may potentially up-regulate enzymes of the urea cycle.

[00407] Six identified compounds, CP-673451, Amuvatinib, Crenolanib, Sunitinib, Amuvatinib, and PDGF, are known modulators of the Platelet-derived growth factor receptor (PDGFR)-mediated signaling pathway. This suggest that modulating signaling molecules, particularly PDGFRs, in the PDGFR-mediated signaling pathway may potentially up-regulate enzymes of the urea cycle.

[00408] Three identified compounds, Pazopanib, Linifanib, Regorafenib, are known modulators of the Platelet-derived growth factor receptor (PDGFR) and Vascular endothelial growth factor receptor (VEGFR) family mediated signaling pathway. This suggest that modulating signaling molecules, particularly PDGFRs, VEGFRs, in the PDGFR and VEGFR-mediated signaling pathway may potentially up-regulate enzymes of the urea cycle.

[00409] Five identified compounds, PF-04929113 (SNX-5422), 17-AAG, BIIB021, HSP-990, Retaspimycin Hydrochloride, are known modulators of the Heat shock protein 90 (HSP90) mediated signaling pathway. This suggest that modulating signaling molecules, particularly HSP90 may potentially up-regulate enzymes of the urea cycle.

[00410] Five identified compounds, GDF2 (BMP9), BMP2, Activin, Nodal and Anti mullerian hormone, Dorsomorphin are known modulators of the transforming growth factor-beta (TGF-B) signaling pathway. This suggest that modulating signaling molecules, particularly TGF-B and/or Bone morphogenetic protein receptor type-1A (BMPRI1A), in the TGF-B signaling pathway may potentially up-regulate enzymes of the urea cycle.

[00411] Five identified compounds, Momelotinib, Baricitinib, GLPG0634, AZD1480, LY2784544, TAK-901, Tofacitinib citrate, are known modulators of the JAK mediated signaling pathway. This suggest that modulating signaling molecules, particularly JAK/STAT may potentially up-regulate enzymes of the urea cycle.

[00412] Three identified compounds, CCCP, BX795, MRT67307 are known modulators of the TBK1/Ikbe mediated signaling pathway. This suggest that modulating signaling molecules, particularly through TBK1 pathway may potentially upregulate urea cycle enzymes.

[00413] Seven identified compounds, AS602801 (Bentamapimod), PF-00562271, BIRB 796, Pamapimod, R1487 Hydrochloride, Bardoxolone, PH-797804, are known modulators of the MAPK mediated signaling pathway. This suggest that modulating signaling molecules, particularly MAPK may potentially up-regulate enzymes of the urea cycle.

[00414] Mubritinib (TAK-165) is a potent inhibitor of HER2/ErbB2 with IC₅₀ of 6 nM in BT-474 cells.

[00415] XL228 is designed to inhibit the insulin-like growth factor type-1 receptor (IGF1R), Src and Abl tyrosine kinases – targets that play crucial roles in cancer cell proliferation, survival and metastasis

[00416] Sunitinib inhibits cellular signaling by targeting multiple RTKs. These include all platelet-derived growth factor receptors (PDGF-R) and vascular endothelial growth factor receptors (VEGF-R). Sunitinib also inhibits KIT (CD117), the RTK that drives the majority of GISTs. In addition, sunitinib inhibits other RTKs including RET, CSF-1R, and flt3

[00417] Lifirafenib (BGB-283) potently inhibits RAF family kinases and EGFR activities in biochemical assays with IC₅₀ values of 23, 29 and 495 nM for the recombinant BRAFV600E kinase domain, EGFR and EGFR T790M/L858R mutant.

[00418] Foretinib (GSK1363089) is an ATP-competitive inhibitor of HGFR and VEGFR, mostly for Met and KDR with IC₅₀ of 0.4 nM and 0.9 nM in cell-free assays. Less potent against Ron, Flt-1/3/4, Kit, PDGFR α/β and Tie-2, and little activity to FGFR1 and EGFR.

[00419] BIIB021 is an orally available, fully synthetic small-molecule inhibitor of HSP90 with K_i and EC₅₀ of 1.7 nM and 38 nM, respectively

[00420] NVP-HSP990 (HSP990) is a novel, potent and selective HSP90 inhibitor for HSP90 α/β with IC₅₀ of 0.6 nM/0.8 nM.

[00421] IPI-504 is a novel, water-soluble, potent inhibitor of heat-shock protein 90 (Hsp90

[00422] Baricitinib is a selective and reversible Janus kinase 1 (JAK1) and 2 (JAK2) inhibitor

[00423] Doramapimod (BIRB 796) is a pan-p38 MAPK inhibitor with IC₅₀ of 38 nM, 65 nM, 200 nM and 520 nM for p38 $\alpha/\beta/\gamma/\delta$ in cell-free assays, and binds p38 α with K_d of 0.1 nM in THP-1 cells, 330-fold greater selectivity versus JNK2.

[00424] Pamapimod is a potent inhibitor of p38 α MAP kinase (IC₅₀ = 14 nM).¹ It displays over 30-fold selectivity for p38 α over p38 β , has no activity against p38 δ or p38 γ , and has limited activity against a panel of 350 other kinases

[00425] Farnesyltransferase inhibitor BMS-214662 inhibits the enzyme farnesyltransferase and the post-translational farnesylation of number of proteins involved in signal transduction, which may result in the inhibition of Ras function and apoptosis in susceptible tumor cells.

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[00426] The results also suggest that expression of urea cycle enzymes may be associated with other signaling pathways, such as the NF- κ B signaling pathway, hypoxia activated signaling pathway, Src signaling pathway, c-MET signaling pathway, cell cycle/DNA damage pathway, Adrenergic receptor-mediated pathway, PAK signaling pathway, MAPK signaling pathway, , farnesyl transferase, EGFR, FGFR and apoptosis. Modulating one of these pathways may also lead to up-regulation of the urea cycle enzymes.

Example 5. Determining genomic position and composition of signaling centers

[00427] A multilayered approach is used herein to identify locations or the “footprint” of signaling centers. The linear proximity of genes and enhancers is not always instructive to determine the 3D conformation of the signaling centers.

[00428] ChIP-seq was used to determine the genomic position and composition of signaling centers. Antibodies specific to 67 targets, including transcription factors, signaling proteins, and chromatin modifications, were selected for validation in HepG2 cells using ChIP-seq. These validated antibodies were used in ChIP-seq for hepatocytes to create a two-dimensional (2D) map. These antibody targets are shown in Table 11.

Table 11. ChIP-seq targets for primary human hepatocytes

Chromatin	Transcription factors		Signaling proteins	
H3K4me3	HNF1A	RNA Pol II	STAT1-JAK/STAT	NR3C1 (glucocorticoid receptor) – nuclear receptor signaling
H3K27ac	FOXA1	ONECUT2	STAT3-JAK/STAT	AR (androgen receptor) - nuclear receptor signaling
H3K4me1	HNF4A	PROX1	TP53-p53, mTOR, AMPK	ESR1 (estrogen receptor) - nuclear receptor signaling
H3K27me3	NROB2	YY1	TEAD 1/2-Hippo	NR1H3 (liver X receptor alpha) - nuclear receptor signaling
p300	FOXA2	CTCF	NF- κ B (p65)-NF- κ B	NR1H4 (farnesoid X receptor) - nuclear receptor signaling
BRD4	CUX2	ONECUT1	CREB1-MAPK	AHR (aryl hydrocarbon receptor) – aryl hydrocarbon signaling
SMC1	HHEX	MYC	CREB2-MAPK	NR1I2 (pregnane X receptor) - nuclear receptor signaling
	ZGPAT		JUN-TLR, MAPK	HIF1a (hypoxia inducible factor) – hypoxia activated signaling
	NR113		FOS-TLR, MAPK	TCF7L2 (TCF4)-WNT
	ATF5		ELK1-MAPK	CTNNB1-WNT
			SMAD2/3-TGF β	RBPI - NOTCH
			SMAD4 - TGF β	SREBP1 – cholesterol biosynthesis
			SMAD1/5/8 - TGF β	SREBP2 – cholesterol biosynthesis
			ETV4 - ERK MAPK	RXR (RA pathway) - nuclear receptor signaling
			RARA - nuclear receptor signaling	NR3C2 (Mineralocorticoid receptor) - nuclear receptor signaling
			NR1I1 (Vitamin D receptor) - nuclear receptor signaling	STAT5 – JAK/STAT
			NR5A2 (liver receptor homolog 1) - nuclear receptor signaling	PPARG – nuclear receptor signaling
			YAP1 – Hippo signaling	PPARA – nuclear receptor signaling

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		TAZ - Hippo signaling	mTOR – mTOR signaling
		MLXIPL – carbohydrate response signaling	GLI3 – Hedgehog signaling
		GLI1 – Hedgehog signaling	ATR – DNA damage response signaling
		WWTR1 – Hippo signaling	

[00429] In the signaling proteins column, the associated canonical pathway is included after the “-”.

[00430] Table 12 shows the chromatin marks, and chromatin-associated proteins, transcription factors, and specific signaling proteins/or factors associated with the insulated neighborhood of each urea cycle-related gene in primary human hepatocytes.

Table 12. ChIP-seq results

Gene	Chromatin	Transcription factors	Specific signaling proteins/or factors
CPS1	H3K27ac, BRD4, p300, SMC1	FOXA2, HNF4A, ONECUT1, ONECUT2, YY1	TCF7L2, ESRA, FOS, NR3C1, JUN, NR5A2, RBPJK, RXR, STAT3, NR1H1, NF-kB, SMAD2/3, SMAD4, STAT1, TEAD1, TP53
OTC	H3K27ac, BRD4	FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, HNF1A	TCF7L2, HIF1a, ESRA, NR3C1, JUN, RXR, STAT3, NF-kB, SMAD2/3, SMAD4, TEAD1
ASS1	H3K27ac, BRD4, p300, SMC1	FOXA2, HNF4A, ONECUT1, MYC, YY1	CREB1, NR1H4, HIF1a, ESRA, JUN, RXR, STAT3, NR1H1, NF-kB, NR3C1, SMAD2/3, SMAD4, TEAD1
ASL	H3K27ac, BRD4, p300	HNF3, HNF4A, ONECUT1, HNF1A, MYC	TCF7L2, CREB1, NR1H4, HIF1a, ESRA, FOS, JUN, RBPJK, RXR, STAT3, NR1H1, NF-kB, NR3C1, SMAD2/3, SMAD4, STAT1, TEAD1, TP53
NAGS	H3K27ac, BRD4, p300	FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, HNF1A	TCF7L2, HIF1a, AHR, ESRA, JUN, RXR, STAT3, NR1H1, NF-kB, NR3C1, SMAD2/3, SMAD4, TEAD1, TP53
ARG1	H3K27ac, BRD4, p300	FOXA2, HNF4A, ONECUT1, ONECUT2, YY1, HNF1A, MYC	HIF1a, ESRA, NR3C1, JUN, RXR, STAT3, NR1H1, SMAD2/3, STAT1, TEAD1
SLC25A15	H3K27ac, BRD4	FOXA2, HNF4A, ONECUT1, ONECUT2, YY1	ESRA, Jun, RXR, NR1H1, NF-kB, NR3C1, SMAD2/3, TP53
SLC25A13	H3K27ac, BRD4, p300, SMC1	FOXA2, HNF4A, ONECUT1, ATF5, ONECUT2, YY1, HNF1A, MYC	TCF7L2, HIF1a, ESRA, NR3C1, JUN, RXR, STAT3, NR1H1, NF-kB, SMAD2/3, STAT1, TEAD1, TP53

Example 6. RNA-seq result validation

[00431] Compounds identified from initial RNA-seq analysis are subjected to validation with qRT-PCR. qRT-PCR is performed on samples of primary human hepatocytes from a second donor stimulated with identified compounds. Compounds are tested at different concentrations and with different cell lots. Fold change in gene expression observed via qRT-PCR is compared to that from RNA-seq analysis. Compounds that cause robust increase in the expression of at least one urea cycle enzyme are selected for further characterization.

Example 7. Disruption of pathway of interest

[00432] Canonical pathways that showed connection with changes in the expression of urea cycle enzymes are perturbed with additional compounds to confirm the involvement of the

pathway in the regulation of urea cycle enzymes. In one embodiment, primary human hepatocytes are treated with additional compounds that target different components in the PDGFR-mediated signaling pathway. In another embodiment, primary human hepatocytes are treated with additional compounds that target different components in the TGF-B signaling pathway. Expression of selected urea cycle enzymes in stimulated hepatocytes is analyzed with RNA-seq as described in Example 1. Hepatic stellate cells are also treated with the same compounds and the effect of the perturbed pathway on gene expression is compared. Changes in binding patterns of the signaling proteins are examined using ChIP. Results are utilized to illustrate gene signaling networks controlling expression of selected urea cycle enzymes and identify additional compounds that modulate selected urea cycle enzymes in the desired direction.

Example 8. Compound testing in other liver cell lines

[00433] In one embodiment, candidate compounds are evaluated in a hepatic stellate cells to confirm their efficacy. Changes in target gene expression in stellate cells are analyzed with qRT-PCR. Results are compared with that from the primary hepatocytes. Compounds that show consistent induction of at least one urea cycle enzyme are selected for further analysis.

Example 9. Compound testing in patient cells

[00434] Candidate compounds are evaluated in patient derived induced pluripotent stem (iPS)-hepatoblast cells to confirm their efficacy. Selected patients have deficiency in at least one of the urea cycle enzymes. Changes in target gene expression in iPS-hepatoblast cells are analyzed with qRT-PCR. Results are used to confirm if the pathway is similarly functional in patient cells and if the compounds have the same impact.

Example 10. Compound testing in a mouse model

[00435] Candidate compounds are evaluated in a mouse model of a urea cycle disorder (e.g., CPS1 deficiency, OTC deficiency, ASS1 Deficiency, ASL deficiency, NAGS deficiency, Arginase deficiency, ORNT1 deficiency, or Citrin deficiency) for *in vivo* activity and safety.

Equivalents and Scope

[00436] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00437] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a

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given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[00438] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” is thus also encompassed and disclosed.

[00439] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00440] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (e.g., any antibiotic, therapeutic or active ingredient; any method of production; any method of use; etc.) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00441] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

[00442] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

Table 13. Motifs for Binding Sites or Signaling Centers

Motif Sequence		SEQ ID NO	Gene symbol	Motif Sequence	SEQ ID NO	Gene symbol
AAAAGTCAA			NR2E1	NAACCGGTTN		TFCP2
AAAATGGCGCCATTTT			E2F2	NACACGTGTN		CLOCK
AAACCACAAA			RUNX1	NACCCGGAAGTAN		ELF3
AAAGTAAACA			FOXA1	NACCGAAACYN		IRF5
AAATGGCGCCATTT			E2F1	NACGCCACGCANN		EGR1
AACAGCTGTT			MSC	NAGGTGTGAAAWN		EOMES

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AACATCTGGA		ZBTB18	NAGGTGTGAAN	TBR1
AACCGGAAGT		ELF1	NAGGTGTGAAN	TBX20
AACCGGAAGT		ETV1	NAGGTGTGAWN	TBX2
AACCGGAAGT		GABPA	NATATGCTAATKN	POU5F1
AATGGCGCCAAA		E2F1	NATGACGTCAYN	JDP2
AATGGCGCCAAA		E2F4	NATGCGGAAGTR	ELF2
AATTGGCCAATTA		LBX2	NATGCGGGTAN	GCM1
ACAGGAAGTG		ERG	NATGCGGGTN	GCM2
ACAGGAAGTG		ETS1	NCCGCCATTNN	YY1
ACATCCTGGT		SPDEF	NCCGTAAACGGN	MYBL1
ACCCTTGAACCC		ZNF524	NCGAAACCGAAACY	IRF8
ACCGAACAAT		SOX17	NCGAAACCGAAACYN	IRF4
aCGCGTc		MBL2	NCGACCACCGAN	ZBTB7B
ACTACAATTCCC		GFY	NCTAATTANN	LHX2
ACTGAAACCA		IRF4	NCTCGTAAAAAN	HOXA13
ACTTTCACTTTC		PRDM1	NCTCGTAAAAAN	HOXB13
AGAAATGACTTCCCT		ZNF528	NCTCGTAAAAAN	HOXC13
AGAGGAAGTG		MAZ	NCTCGTAAAAAN	HOXD13
aGATAAG		SLC6A1	NGATGACGTCATCR	FOS
AGGCCTAG		ZNF711	NGCGACCACCNN	ZBTB7A
AGGCCTGG		ZFX	NGGCACGTGCCN	HES5
AGGTCTCTAACC		PRDM14	NGGCACGTGCCN	HES7
AGGTGTGA		MGAM	NGTCGTAAAAAN	HOXC12
AGGTGTGA		TBX1	NGTCGTAAAAAN	HOXD12
AGGTGTGA		TBX15	NKCACGTGMN	BHLHE40
AGGTGTGA		TBX4	NKCACGTGMN	BHLHE41
AGGTGTGA		TBX5	NNAAATGGCGCCAAAAANN	E2F3
AGGTGTGAAAAAAGGTGTGA		TBX1	NNAAATGGCGCCATTNN	E2F3
AGGTGTGAAGGTGTGA		TBX20	NNAACCGTTNN	MYBL1
ATaacATG		SLC6A11	NNAACCGTTNN	MYBL2
ATAGTGCCACCTGGTGGC CA		CTCF	NNACGCCCACGCANN	EGR3
ATCAATAACATTGAT		SOX15	NNACGCCCACGCANNN	EGR4
ATCAATTGCAGTGAT		SOX10	NNATGACGTCATNN	ATF7
ATCACGTGAT		MLX	NNCACCTGCN	TCF3
ATCACGTGAT		MLXIPL	NNCACCTGNN	FIGLA
ATGAATAACATTCAT		SOX15	NNCACCTGNN	MESP1
ATGAATTGCAGTCAT		SOX10	NNCACCTGNN	TCF3
ATGACGTCAT		JUN	NNCACCTGNN	TCF4
ATGACTCAT		BACH1	NNCACGTGNN	HEY1
ATGACTCATC		FOS	NNCACGTGNN	MAX
ATGCCCTGAGGC		TFAP2A	NNCACGTGNN	MNT
ATTGCGCAAC		CEBPA	NNCACGTGNN	TFE3
CAAGATGGCGGC		YY1	NNCATATGNN	BHLHA15
CACCGAACAAT		SOX6	NNCATATGNN	NEUROD2
CCAAT		NFYA	NNCCGCCATNW	YY2
CCAAT		NFYB	NNGCAACAGGTGGNN	SCRT1
CCAAT		NFYC	NNGCAACAGGTGN	SCRT2
CCATGCCGCCAT		YY2	NNGGAAGTGCTTCCNN	ETV7
CCCCGCGC		SLC13A4	NNGGATTANN	DMBX1
CGCAGCTGCG		NHLH1	NNGGATTANN	DPRX
CGCGAAAa		VANGL2	NNMCGCCCACGCANN	EGR2
CGGACACAAT		FOXK1	NNMCGCCCACGCANNN	EGR4
CGGTTGCCATGGCAAC		RFX1	NNNNNTGCTGAN	MAFK
CGGTTTCAAA		CHR	NNNNNTGCTGAN	NRL
CGTGGGTGGTCC		GLI3	NNNTGGCGCCANNN	E2F1

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CTAATTAG		PAX4	NNRRAAAGGAAACCGAAACT N	IRF3
CTGCGCATGCGC		NFE2L1	NNTAAACGNN	BARHL2
CTTCGAG		XBP1	NNTAATCCGATTANN	OTX1
GAAAGTGAAAGT		IRF1	NNTAATCCNN	GSC
GAGGTCAAAGGTCA		NR2C2	NNTAATCCNN	GSC2
GATGACGTCATC		XBP1	NNTAATTANN	HOXD8
GATGACTCAGCA		NFE2	NNTAATTGNN	BARHL2
GATTGCATCA		APEH	NNTAATTRNN	HESX1
GCACTTAA		ISL2	NNTCCAGATGTKN	ZBTB18
GCAGCCAAGCGTGACC		PAX5	NNTGCCAAN	NFIX
GCTAATCC		CRX	NNTGCCAANN	NFIA
GCTTTTCCCA		ZNF75A	NNTTTTGGCGCCAAAANN	E2F2
ggACCCt		NRG1	NNTTTTGGCGCCAAAANN	E2F3
GGCCATAAATCA		HOXA9	NNYAATTANN	ALX3
GGGGGTGTGTCC		KLF10	NNYAATTANN	DLX1
GGGGTCAAAGGTCA		RXRA	NNYAATTANN	EMX1
GGGGTCAAAGGTCA		RXRB	NNYAATTANN	EMX2
GGGGTCAAAGGTCA		RXRG	NNYAATTANN	EN1
GGTCACGTGA		USF1	NNYAATTANN	EN2
GGTCCCTAGGGA		EBF1	NNYAATTANN	ESX1
GTAAACA		FOX11	NNYAATTANN	GBX1
GTAAACA		FOXO4	NNYAATTANN	GBX2
GTAAACA		FOXO6	NNYAATTANN	HOXA1
GTAAACAA		FOXO1	NNYAATTANN	LBX2
GTAAACATGTTTAC		FOXO1	NNYAATTANN	MIXL1
GTAAACATGTTTAC		FOXO3	NNYAATTANN	MNX1
GTAAACATGTTTAC		FOXO4	NNYAATTANN	PRKRA
GTAAACATGTTTAC		FOXO6	NNYMATTANN	GSX1
GTAATAAAA		HOXD12	NNYMATTANN	GSX2
GTACCTACCT		ZNF784	NNYMATTANN	HOXA2
GTCACGTG		RBPJ	NNYMATTANN	HOXB2
GTCACGTGAC		ARNTL	NNYMATTANN	HOXB3
GTCACGTGAC		BHLHE41	NNYMATTANN	HOXB5
GTCCCCAGGGGA		EBF1	NNYMATTANN	HOXD4
GTGCGCATGCGC		NKRF	NNYTAATTANN	VSX1
GTGGGCCCCA		ZNF692	NNYTTCCGGGAARNR	ETV7
TCACACCTAGGTGTGA		T	NRAAAGTGAAAGTGN	PRDM1
tCACGTG		RBPJ	NRCACCTGNN	ID4
TGACCTTTAAAGGTCA		NR4A2	NRCAGGTGN	SNAI2
TGATGACGTCATCA		BATF3	NRCATTCCWN	TEAD4
TGCCACGTGGCA		CREB3L1	NRGATAATCYN	DPRX
tGCTGGT		ACE2	NRGTCCAAAGTCCANY	HNF4A
TGGCAGTTGG		MYBL1	NRTTACGTAAYN	DBP
TTACGTAA		GMEB2	NRTTACGTAAYN	EPAS1
TTACTAA		ARRB1	NRYYTCCGGH	FLII
TTgccATggCAAC		RFX1	NSTAATTANN	HOXA3
tTgTTTAc		FOXO1	NSTAATTANN	MEOX1
TTTAAAGGTCA		NR4A2	NSTAATTANN	MEOX2
TTTCCCCACAC		FOXO3	NTAATCCN	OTX1
TTTCCCCACACG		FOXO1	NTAATCCN	OTX2
TTTCCCCACACG		FOXO4	NTAATCCN	PITX1
TTTCCCGCCAAA		E2F8	NTAATTAN	LMX1A
TTTGGCGCCAAA		E2F1	NTCAAGGTCAN	ESRRA
TTTGGCGCCAAA		E2F4	NTGACAGN	MEIS3P1
TTTTCCATGGAAAA		NFATC1	NTGACAN	MEIS1
AAAAAGCGGAAGTN		SPI1	NTTAATCCN	PITX1

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AAAAATGCGCCAAAAANN		E2F2	NWAACCACADNN		RUNX2
AAAGATCAAAGGRWW		LEF1	NYAATTAN		DLX2
AACCCGGAAGTN		ELF1	NYAATTAN		DLX3
AACCCGGAAGTR		ELF1	NYAATTAN		DLX4
AACCCGGAAGTR		ELF4	NYAATTAN		DLX5
AACCGTTAACGGNN		MYBL2	NYAATTAN		DLX6
ACCCGGAAGTN		ELF5	NYAATTAN		EN1
ACCGGAAGTN		ELK1	NYAATTAN		ISX
ACCGGAAGTN		ELK3	NYAATTAN		LHX9
ACCGGAAGTN		ERF	NYAATTAN		LMX1B
ACCGGAAGTN		ETV3	NYAATTAN		MSX1
ACCGGAAGTN		ETV4	NYAATTAN		MSX2
ACCGGAAGTN		FEV	NYAATTAN		PRRX1
ACCGGAAGTN		GABPA	NYAATTAN		PRRX2
ACCGGAARYN		ETS1	NYAATTAN		RAX2
ACCGGAARYN		ETV1	NYAATTAN		SHOX
ACCGGAARYN		FLI1	NYAATTAN		SHOX2
AGATAAN		GATA1	NYAATTAN		UNCX
AGATAANN		GATA3	NYMATAAAN		CDX1
AGATAANN		GATA4	NYMATAAAN		CDX2
AGATAANN		GATA5	NYMATTAN		HOXA7
AGATAASR		GATA3	NYMATTAN		NKX6-1
AGGTGTGANN		TBR1	NYMATTAN		NKX6-2
AGGTGTNR		TBX3	NYMATTAN		PDX1
CCAATAAAAN		HOXA13	NYNATTAN		BARX1
CCAATAAAAN		HOXB13	NYNATTAN		BSX
CCAATAAAAN		HOXC13	NYTAATCCN		PITX1
CCGAAACCGAAACY		IRF5	NYTAATCCN		PITX3
cTCGAGG.		XBP1	NYTAATTARN		LHX6
gCTTCCw		NR3C1P1	NYTAGTTAMN		HMBX1
GGCGGGAAAH		E2F4	NYTGTGTTACHN		FOXP1
GGCGGGGAARN		E2F6	RACATATGTY		NEUROG2
GHCACGTG		CLOCK	RAGGTCAAAAGGTCAAKN		RARA
GNCACGTG		ARNTL	RATAAAAR		CPEB1
GTAAACAW		FOXO3	RCGGACACAATR		FOXG1
TCAAGGTCAWN		ESRRB	RGGATTAR		GSC
tgCACCy		FAM64A	RGGcAm		EGLN2
tgCATTT.		CST6	RGGGCACTAACy		ZNF264
tGCTGg..		SWI5	RRGGTCAAAGTCCRNN		HNF4A
TGCTTTCTAGGAATTMM		BCL6B	RRGGTCAN		NR2F1
TGGGGAAGGGCM		ZNF467	RRGGTCATGACCYY		RXRA
TTGACAGS		MEIS2	RRGGTCATGACCYY		RXRG
TTTTCCCGCCAAAW		E2F7	RTCACGTGAY		SREBF2
aaa.GTAAACAa		FOXG1	RTCACGTGAY		TFEB
AACAATAMCATTGTT		SOX15	RTCACGTGAY		TFEC
AACAATANCATTGTT		SRY	RTCACGTGAY		USF1
AACAATKNYAGTGTT		SOX8	RTGCCMNNN		HIC2
AACAATNNCATTGTT		SOX18	SCACGTGS		MYCLP1
AACAATNNNAGTGTT		SOX10	SYMATTAN		HOXA6
AACAATNNNCATTGTT		SRY	VCAGCTGBNN		TCF12
AACA ~~V~~ BTGTT		MYF6	WATGCGCATW		POU5F1
AACCGGWNNNWCCGGTT		GRHL1	WRCGTAACCACGYN		GMEB2
AAGAACATWHTGTTC		PGR	YGCGCATGCGCN		NFE2L1
AAGGTCA ~~NN~~ NAAGGTCA		ESRRG	yGCGGck.		SULT1A1
AANTAGGTCAGTAGGTCA		RORB	YRATTGCAATYR		LHX6
AARGGTCAAAAGGTCA		RARB	YTAATTAN		VAX1
ACAATANCATTG		SOX14	YTAATTAN		VAX2

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ACCGGAWATCCGGT		ERG	YTAATTAN		VSX1
ACCGGAWATCCGGT		FLII	YTAATTAN		VSX2
ACCGTTARRACCGTTA		MYBL2	...a...gTAAACAa		FOXG1
ACTACTAwwwTAG		TMEM50 A	.gCsGgg		SLC13A4
ACVAGGAAGT		ELF5	DATGASTCAT		BATF
AGATGKDGAGATAAG		GATA3	kGmCAGCGTGTC		TRIM3
AGGTCANNNNTGACCT		NR4A2	NAACAATTKCAGTGTT		SOX4
AGGTGTGAANTTCACACC T		TBX15	NATTTCCNGGAAAT		STAT1
AGGTGTGAAWTTCACACC T		TBX1	NCCGCCATNNT		YY2
AGGTGTGAWWWTCACAC CT		TBX20	NCYAATAAAAA		HOXD13
AGTGTTAACAGARCACT		ZSCAN16	NGYAATAAAAA		HOXD11
ANACAGCTGC		TCF3	NNCATCCCATATANTC		ZNF410
ANCAGCTG		KRT7	NNNGCATGTCCNGACATGCC		UVRAG
ARGGTCAAAAGGTCA		RARA	NNNGMCACGTCATC		XPB1
ATAAACAAWGTAAACA		FOXG1	NRAAGGTCAANNNNRGGTCA		RORA
ATCAATNNCATTGAT		SOX18	NTGCCACGTCANCA		CREB3L1
ATGAATKNYAGTC		SOX8	NYAATTTAAANNNYAATTA		MSX1
ATGAATWYCATTTCAT		SOX18	NYAATTTAAANNNYAATTA		MSX2
ATGASTCAT		JDP2	RACATGTYNRRACATGTC		TP63
ATGCGGGYRCCCGCAT		GCM1	RAGGTCANTCAAGGTCA		ESRRA
ATGMATAATTAATG		POU4F1	RAGGTCANTCAAGGTCA		ESRRG
ATTTCTNAGAAA		STAT5A	RAGTCAANAAGTCA		NR2E1
AVCAGGAAGT		EHF	rCGGC...rCGGC		SULT1A1
CACGTGNNNNNCACGTG		MAX	RGTTCRNNNRGTTC		NR1I2
CGatAa..sC		APEX1	RMATWCCA		TEAD3
CGG.....cCg		LGALS4	rracGCsAaa		VANGL2
CGGas.rsw.y.s.CCGa		LGALS4	RRGGTCANNNRRGGTCA		RARG
CTTGGCACNGTGCCAA		NF1	RTAAACAATAAAYA		FOXJ2
CWGGCGGGAA		E2F1	RTAAACARTAAACA		FOXJ3
GACCCCCCGCGNNG		GLIS2	RTAAACAWMAACA		FOXJ2
GACCCCCCGCGNNG		GLIS3	RTAAAYA		FOXJ2
GAGSCCGAGC		ZNF519	RTAAAYA		FOXJ2
GARTGGTCATCGCCC		ZNF669	RTAAAYA		FOXJ2
GCGACCACNCTG		GLI2	RTAAAYAAACA		FOXJ2
ggGTGca.t		FAM64A	rTGTAcGGrT		FHL1
GGGTTTTGAAGGATGART AGGATT		ZFP3	RTGTAAAYGTAGATTAAG		ZNF232
GGNTCTCGCGAGAAC		ZBTB33	WRTAAAYAAACAA		FOXJ2
GGTGTGANNTCACACC		TBX21	WTTCGAAYG		HSFY2
GGTGTGAWATCACACC		TBX21	YACGTAACNKACGTA		GMEB2
GNCCACGTGG		MYC	yGGCGCTAyca		SNTB1
GSCTGTCACTCA		PBX1	YTGGCANNTGCCAA		NFIX
GTCACGCTCNCTGA		PAX5	YTGGCANNNTGCCAA		NFIX
GTGGTCCCGGATYAT		SPDEF	..ACCCRaCMY		RAP1A
GYAATAAAA		HOXC12	.iGrTAGCGCCr...		SNTB1
TAATTRNNYAATTA		GBX2	KCTAWAAATAGM		MEF2A
TAATTRSNYAATTA		EN1	MCCATATAWGGN		SRF
TAATYNAATTA		PROP1	NAAACGATNNAN		HOMEZ
TAATYRATTA		PAX3	NAACAATANCATTGTTN		SOX2
TAATYRATTA		PAX7	NAACAATKNYAGTGTTN		SOX8
TAATYTAATTA		PRRX1	NAACAATKNYAGTGTTN		SOX9
TAATYYAATTA		PHOX2A	NAACAATNNNATTGTTN		SOX2
TAATYYAATTA		PHOX2B	NAACCGCAAACCRCAN		RUNX2
TAAYYNAATTA		PROP1	NAACCGCAAACCRCAN		RUNX3

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TATGCWAAT		POU2F3	NAACCRCAAN		RUNX3
TATGCWAAT		POU3F4	NAAGACGYCTTN		PROX1
TATGCWAAT		POU5F1B	NAANCGAAASYR		IRF2
TCAATANCATTGA		SRY	NAASATCAAAGN		TCF7L1
TCAATNNCATTGA		SOX14	NACAYATGNN		TCF15
TCAATNNNATTGA		SOX21	NACTTCCGSCGGAARMN		ELK1
TCACACCTNNNNAGGTGT GA		EOMES	NAGGTCAMSRTGACCTN		ESR1
TGAATANCATTCA		SRY	NAMCCGGAAGTR		ELF2
TGACAGSTGTCA		MEIS3P1	NATCAATANCATTGATN		SOX2
TGACAGSTGTCA		PKNOX1	NATCAATKNYAGTGATN		SOX8
TGACAGSTGTCA		PKNOX2	NATCAATKNYAGTGATN		SOX9
TGACAGSTGTCA		TGIF1	NATCAATNNNATTGATN		SOX2
TGACAGSTGTCA		TGIF2	NATGAATKNYAGTCATN		SOX9
TGACAGSTGTCA		TGIF2LX	NATGASTCATN		NFE2
TGCACACNCTGAAAA		ZSCAN4	NCACTTNAN		NKX2-8
TGGAACAGMA		ZNF189	NCCACTTRAN		NKX2-3
TRNGTAAACA		FOXA1	NCCGGAWATCCGGN		ERG
TTaGTmAGc		YAP1	NCCGGAWRYRYWTCCGGN		ETS1
TTCKKNAGAA		STAT6	NCCGGNNNNNNCCGGN		TFCP2
TTCGAANNNTTCGAA		HSFY2	NCGAAANYGAAACY		IRF7
TTCTAGAANNNTTC		HSF1	NCGACCACCNAN		ZBTB7C
TTCTAGAANNNTTC		HSF2	NCTAWAAATAGM		MEF2D
TTCTAGAANNNTTC		HSF4	NGACCCCCACGANGN		GLIS1
TTGACAGSTGTCAA		MEIS2	NGAGGTCANNGAGTTCANN		CYP27B1
TTTCAAGGCYCCC		PRDM4	NGCCNNNNNGGCN		TFAP2A
TTTCCAYNRTGGAAA		NFATC1	NGCCNNNNNGGCN		TFAP2B
TTTCCCCACACRAC		FOXO6	NGCCNNNNNGGCN		TFAP2C
TTTTATKRGG		HOXB13	NGCCNNNNNGGCN		TFAP2C
AAAAAGMGGAAGTN		SPIB	NGGGGAWTCCCN		NFKB1
AAAAAGNGGAAGTN		SPIC	NGGGGAWTCCCN		NFKB2
AACCGGAARTR		ETV2	NGTCGTWAAAN		HOXC11
ACCGGAARTN		ELK4	NGTCGTWAAANN		HOXA10
ACCGGAARTN		ERG	NHAACBGYYV		MYBL2
ACCGGAWATCCGGN		FLI1	NMCGCCCMCGCANN		EGR1
ACCGGAWGYN		ETV5	NMCRATTAR		VENTX
AGGTCANTGACCTN		NR1H4	NNAAAATCRATANN		ONECUT3
AMCCGGATGTN		SPDEF	NNAAAATCRATAWN		ONECUT1
AVYTATCGATAD		ZBED1	NNAAAATCRATAWN		ONECUT2
AWCGAAACCGAAACY		IRF9	NNATGAYGCAATN		ATF4
CCAAT.a.		NFYA	NNCAMITTAANN		HMX1
CCCATWAAAN		HOXC10	NNCAMITTAANN		HMX2
CCGGAASCAGGAAGTN		ETV6	NNCAMITTAANN		HMX3
CCWGGAATGY		TEAD2	NNMCCATATAWGGKNN		SRF
CCWGGAATGY		TEAD4	NNNNTGCTGASTCAGCANNNN		MAFG
CGSGTAA.		VANGL1	NNNNTGCTGASTCAGCANNNN		MAFK
CTCGRACCCGTGCN		ZNF524	NNRTGACGTCAFCN		CREB3
CTTCCCCMYAACACKNN		ZNF282	NNTAATCCGMITTANN		OTX2
CYAATAAAAN		HOXD13	NNTCCCNNGGGANN		EBF1
GAAASYGAAASY		IRF2	NNTNATTANN		EVX1
GAATGACACRGCGN		FOXB1	NNTNATTANN		EVX2
GAGCCTGGTACTGWGCCT GR		ZNF322	NNYATGMATAATTAATN		POU1F1
GGCVGTTR		MYB	NRGGTCANNGGTCAN		NR2F1
GNCACGTGYN		HEY2	NRMATWCCWN		TEAD1
GTAATWAAAN		HOXC10	NRNWAAAYRTTKNYN		FOXD2
GTCACGCNNMATTAN		PAX3	NRNWAAAYRTTKNYN		FOXD3
GTCGTWAAAN		HOXC10	NRRTAATNATTAACN		HNF1A

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GTCGTWAAAN		HOXD11	NRRTAATNATTAACN		HNF1B
GTTAATNATTAAY		HNF1B	NRRTACRTAAYN		NFIL3
TAATCRATAN		CUX1	NRRTACRTAAYN		TEF
TAATCRATAN		CUX2	NSCCNNNGGSN		TFAP2A
TRTTTACTTW		FOXM1	NSCCNNNGGSN		TFAP2B
TSAAGGTCAN		ESRRG	NSCCNNNGGSN		TFAP2C
TTACGYAM		GMEB2	NSCCNNNNNGGSN		TFAP2A
TTGGCANNNTGCCAR		NFIA	NSCCNNNNNGGSN		TFAP2B
TTGGCANNNTGCCAR		NFIB	NSCCNNNNNGGSN		TFAP2C
A.yTrAAt		ARRB1	NSCTMATTAN		POU6F2
AACAATNNNAKTGTT		SOX21	NTAATTANNNTAATTAN		MEOX2
AACAATNNNAKTGTT		SOX21	NTAATYNRATTAN		ALX3
AACAATNNNAKTGTT		SOX7	NTAATYNRATTAN		ALX4
AAGGKGRCGCAGGCA		ZNF165	NTAATYNRATTAN		UBA2
ACCCTmAAGGTyrT		ZNF569	NTAATYTAATTAN		ISX
ACNGTTAAACNG		MYBL1	NTAATYTAATTAN		UNCX
AkGmCACGTA		TRIM3	NTAAYYNAATTAN		DRGX
AMCATATGKT		OLIG2	NTAAYYYAATTAN		ALX1
AMCATATGKT		OLIG3	NTATCGCGAYATR		ZBED1
ANCATATGNT		BHLHE23	NTATGCWAATN		POU2F2
ANCATATGNT		OLIG1	NTATYGATCH		ONECUT1
ANRTAAAYAAACA		FOXC1	NTGAATNNCATTAN		SOX14
ARRGGTCANNRRGGTCA		RARA	NTGAATNNNATTAN		SOX2
AWCAGCTGWT		TFAP4	NTGAATWNCATTAN		SOX2
AWNTAGGTCATGACCTAN WT		RORB	NTGACCTNNNNNAGGTCAN		THRB
CCCGCNTNNNRCGAA		CENPB	NTGCCACGTCAAYCN		CREB3
cCr.RTerGG		ASCL1	NTGCTGANTCAGCRN		MAFK
CGTRNAAARTGA		MSC	NTGCTGASTCAGCAN		MAFF
CNNBRGCGCCCCCTGSTG GC		CTCFL	NTGMATAATTAATGAN		POU4F3
GACCACMCACNNNG		GLI2	NTMATCATCATTAN		MEOX2
GCCMCGCCCMC		AZF1	NTTRCGCAAY		CEBPB
GCCMCGCCCMC		KLF16	NTTRCGCAAY		CEBPD
GCCMCGCCCMC		PSG1	NTTRCGCAAY		CEBPE
GNCACGTGNC		HES1	NTTRCGCAAY		CEBPG
GRGGTCAAAAAGGTCANA		RARG	NWACAYRACAWN		IRX2
GRGGTCAAAAAGKTCAC		RARG	NWACAYRACAWN		IRX5
GRGTTCAANNRRGTTCA		CYP27B1	NWTATGCWAATN		POU2F1
GTCWGTCTGYCTCT		ZNF317	NWTATGCWAATTW		POU3F3
GTigyCATgG.aAc		RFX1	NYAATTARNNNYAATTAN		PDX1
GYCATCMATCAT		HOXA2	NYTMATTANN		NOTO
TAATTAGYRYTRATTA		LHX6	RACCGTTAAACNGYY		MYBL2
TAATTRCYAATTA		LHX9	RCATTCCNNRCATTCCN		TEAD3
TGAATNNNAKTCA		SOX21	RCTAWAAATAGM		BORCS8- MEF2B
TGAATRTKCAGTCA		SOX10	RRGGTCANNNGGTCAN		NR2F1
TGGKCTARCTCGA		ZKSCAN3	RTMATTAN		HOXA4
TTCya.....TTC		HSF1	SCTMATTANN		POU6F2
tttCC.rAt..gg		SRF	WAACCRCAN		RUNX2
TTTCGCNTGGCNNGTCA		ZBTB49	WGTAAYAN		FOXBI
TWCCCAAYAATGCATTG		ZNF143	WTATGCWAATNW		POU3F1
AGATSTNDNDSAGATAA SN		GATA3	YAATTANNCTAATTR		LHX2
ARGAGMCAAAATGW		ZNF675	YMATTARYTAATKR		EMX1
ATTTCCAGVAKSCY		ZNF143	YMATTARYTAATKR		EMX2
GGmraTA.CG		APEX1	YTCACACCTNNAGGTGTGAR		TBX1
AACAATRTKCAGWGTT		SOX10	DGGGYGKGGC		KLF5

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AACAATRTKCAGWGTT		SOX8	NGACCACMCACGWNG	GLI3
AACAATRTKCAGWGTT		SOX9	NGCCACGCCCMCNT	SP6
ADGGYAGYAGCATCT		PRDM9	NTGMATAATTAATKAG	POU4F2
AGGTGTKANGGTGTTSA		TBX20	NWTATGCWAATKAG	POU1F1
AGGTGTKANNTMACACCT		MGAM	rGAA..TlctrGAA	HSF1
AGGTGTNANWWNTNACA CCT		TBX4	RGGTCANNNARRGGTCA	RARA
AGGTGTNANWWNTNACA CCT		TBX5	RGKGGGCGKGGC	SP6
ATCAATRTKCAGWGAT		SOX9	RRGGTCANNNARAGGTCA	RARG
ATCRATNNNNNATCRAT		CUX1	rTCAyt...Acg	MSC
ATCRATNNNNNATCRAT		CUX1	rTGTayGGrtg	FHL1
ATCRATNNNNNATCRAT		CUX2	SAAGGTCANNTSAAGGTCA	ESRRA
ATGAATRTKCAGWCAT		SOX9	sc.GC.gg	EGLN2
CCTCATGGTGYCYTWYTC CCTTGTG		ZNF41	SMCAGTCWGAKGGAGGAGGC	ZSCAN22
GACCMCCYRMTGNG		ZIC1	WTATGCWAATKA	POU3F2
gcsGsg..sG		SLC13A4	.rTCAyt.y..ACG.	MSC
GGMTNAKCC		RHOXF1	MACCTTCYATGGCTCCCTAKT GCCY	ZNF16
GGTGTNANWWNTNACAC C		TBX2	NAACAATKNYAKTGTTN	SOX7
GGYGTGANNNTCACRCC		MGAM	NANCATATGNTN	BHLHE22
GRTGMRGAGCC		ZNF415	NATGAATKNYAKTCATN	SOX7
TAAWYGNNNNNTAAWYG		BARHL2	NATGGAAANWWWWTTTYCM N	NFATC1
TGAATRTKCAGWCA		SOX8	NCAGNAAGAMGTAWMM	SPDEF
TGMATWWWTNA		POU3F4	NGCGCCMYCTAGYGGTN	CTCF
tGyayGGrtg		RAP1A	NGNTCTAGAACCNGV	ZBTB12
tklCC..wTl.GGAAA		SRF	NGTCACGCWTSRNTGNYY	PAX2
TNACACCTNNNAGGTGTN A		TBX21	NGTCACGCWTSRNTGNYY	PAX5
TRGGTYASTAGGTCA		NR1D2	NGTTNCCATGGNAACN	RFX3
TYTCACACCTNNNAGGTG TGARA		TBX1	NGTTNCCATGGNAACN	RFX4
AAACYKGTTWDACMRGT TTB		GRHL2	NGTTRCCATGGYAACN	RFX2
GACCCCCYGYTGNGN		ZIC3	NGTTRCCATGGYAACN	RFX5
GACCCCCYGYTGNGN		ZIC4	NGYAATWAAAN	HOXA10
TTTYACGCWTGANTGMNY N		PAX6	NGYAATWAAAN	HOXC11
ACCYTmArGGT.rTG		ZNF569	NMCMCGCCCMCN	SP8
CNATTAAAWANCNATTA		BARX1	NNGNNACGCCCMYTTTNN	KLF13
TAGAYRAYTGMANGAA		ZNF713	NRTMAATATTKAYN	FOXC1
TWGHWACAWTGTWDC		DMRT1	NRTMAATATTKAYN	FOXC2
GNCTGTASTRNTGBCTCHT T		ZNF382	NTAATTRGYAATTAN	HESX1
....gTAAACAa		FOXO1	NTGACCTNATNAGGTGAN	THRA
KCCACGTGAC		NPAS2	NTGACCTNATNAGGTGAN	THRB
MCGCCACGCA		EGR2	NTGTTTACRGTAAYAN	FOXI1
NACCCGGAAGTA		EHF	NTRAKCCN	RHOXF1
NAGGTGTGAA		TBX21	NTRAYNTAATCAN	DUXA
NATGCGGGTA		GCM1	NWRGCMCGCCCMCTNN	SP4
NCCACGTG		MYC	NWTRMATATKYAWN	POU2F1
NCCACTTAA		NKX3-1	NWTRMATATKYAWN	POU2F2
NCCACTTAA		NKX3-2	RCATTCCNNRCATWCCN	TEAD1
NCCCCCCCAC		ZNF740	RTGGAAAANTMCNN	NFAT5
NCGAAACCGAAACT		IRF8	SGTTGCGYARGCAACS	RFX2
NNCACGTGCC		HEY2	SGTTGCGYARGCAACS	RFX3

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NNGCGGAAGTG		ETV7	SGTTGCGYARGCAACS	RFX4
NTGGGTGTGGCC		KLF1	SGTTRCCATRGCAACS	RFX5
NTTTCAGGAAA		STAT4	SSYTAATCGRWAANCGATTAR	VENTX
rACGCGt		MBL2	w.TTRamkAR	SPTLC2
rCACAAT		MAT1A	WAWRTAAAYAWW	FOXC2
RCCGGAAGTG		ETS2	WNWGTMAATATTRACWNW	FOXB1
RRGGTCAAAAGGTCA		NR2F6	WRWGTMAAYAN	FOXB1
RRGGTCAAAGGTCA		HNF4A	WRWRTMAAYAW	FOXC1
RRGGTCAAAGGTCA		NR2C2	WTAATKAGCTMATTAW	POU6F2
RRGGTCAAAGGTCA		NR2F6	ymtGTmTytAw	SPTLC2
RRGTCCAAAGGTCAA		HNF4A	NGTGTTCAVTSAGCGKAAA	PAX6
RTAAACA		FOXP3	NWTGMATAAWTNA	POU3F2
RTAAACAA		FOXJ2	SGTCACGCWTGANTGMA	PAX1
RTAAACAA		FOXJ3	SGTCACGCWTGANTGMA	PAX9
RTAAACATAACA		FOXJ3	VAGRACAKNCTGTBC	PGR
RTCATGTGAC		MITF	WAWNTRGGTYAGTAGGTCA	RORA
VDTTTCCC GCCA		E2F7	WTGMATAAWTNA	POU3F1
WACCCGGAAGTA		ELF3	WTGMATAAWTNA	POU3F3
WDNCTGGGCA		ZNF416	WTRMATATKYAW	POU2F3
wwwsyGGGG		VPS4B	WTRMATATKYAW	POU3F3
YGTCTAGACA		SMAD3	WTRMATATKYAW	POU5F1B
yTGACT		ASCL1	YTKGATAHAGTATTCTWGGTN GGCA	ZNF136
.gcTgAcTAA.		YAP1	NNCNGTTNNNACNGTTN	MYBL1
DNNNGGTCANNNH		NR2F1	NRGNACANNNTGTNCYN	NR3C2
HAATAAAGNN		PABPC1	NRGWACANNNTGTWCYN	NR3C1
HNHNAGGTGTGANHH		TBX20	NTGACCTYANNTRAGGTCAN	THRB
kGCTGr		SWI5	RAANCGAAAWTCGNTTY	IRF7
KSYGGAAGTN		ETV6	RRGWACANNNTGTWCYY	AKR1B1
NAAACCGGTTTN		GRHL1	WAACCRCAAWAACCRCAN	RUNX2
NAACAATRN		SOX9	WAACCRCAAWAACCRCAN	RUNX3
NAACCGCAAN		RUNX3	NNTCRCACNTANGTGYGANN	TBX19
NAACCGGTTN		GRHL1	YwTTkcKkTyckgykky	AZF1

Table 14. Motifs for Binding Sites or Signaling Centers

Motif Sequence	SEQ ID NO	Complex_name
ACCGGAAATGA		HOXB2:ETV1
CCATAAATCA		PBX4:HOXA10
TAAACAGGATTA		FOXJ2:PITX1
TGTCAATTA		MEIS1:DRGX
ACCGGAAACAGCTGNN		TFAP4:FLI1
ACCGGAAATGAN		HOXB2:ETV4
AGGTGTTAATKN		HOXB2:TBX3
AGGTGTTGACAN		MEIS1:EOMES
CTCGTAAAATGYN		TEAD4:HOXB13
GAAAACCGAAAN		FLI1:FOXJ1
GGAATGCGGAAGTN		TEAD4:ELF1
GGAATGTTAATTR		TEAD4:DRGX
GTGCGGGCGGAAGTN		GCM1:ELF1
TCACACCGGAWRY		ETV2:EOMES
TGTTGCCGGANN		FOXO1:ELK1
ACAAWRSNNNNNYMATTA		HOXB2:SOX15
ACAAWRSNNNNNYMATTA		HOXB2:SOX15
ACAAWRSNNNNNYMATTA		HOXB2:SOX15
AGGTGTKAAGTCGTAAA		HOXD12:TBX21
CAGCTGNNNNNNNNNTGCGGG		GCM1:FIGLA
CAGCTGNNNNNNNNNTGCGGG		GCM1:FIGLA
CAGCTGNNNNNNNNNTGCGGG		GCM1:FIGLA

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CAGCTGNNNNNNNTGCGGG	GCM1:FIGLA
CCGGAANNNNNNCACGTG	ETV5:HES7
GGAATGNNNNNYTAATTA	TEAD4:ALX4
GGATTANNNNNNNNTGCGGG	GCM1:PITX1
GGATTANNNNNNNNTGCGGG	GCM1:PITX1
GGATTANNNNNNTGCGGG	GCM1:PITX1
GGTGTGNNNNNCACGTG	CLOCK:TBX3
TAATKNNNNNNNNAAGGTCA	HOXB2:ESRRB
TAATKRNGGATTA	HOXB2:PITX1
TAATKRNNNNGCAAC	HOXB2:RFX5
TAATKRNNNNGGATTA	HOXB2:PITX1
TAATKRNNNNGGATTA	PITX1:HOXA3
TAATKRNNNNGCAAC	HOXB2:RFX5
TAATKRNNNNNNAAGGTCA	HOXB2:ESRRB
TAATKRNRNGGATTA	HOXB2:PITX1
TAATTANNNNNNCACGTG	CLOCK:EVX1
TGACANNNNNTCATTA	MEIS1:EVX1
TGACANSNTAATTG	MEIS1:DLX3
TMACACCGGAAG	ERF:TBX21
TNTCACACCGGAAAT	ERF:EOMES
ACCCGCANCCGGAAGN	GCM1:ELK3
ACCGGANNTACGCNNNNNYR	ETV2:PAX5
AGGTGNTAATKR	MGA:DLX3
AGGTGNTAATKW	MGA:EVX1
AGGTGNTAATTR	MGA:DLX2
ATTTGCATNACAATRN	POU2F1:SOX2
CACGTGNNNGCGGGY	GCM1:MAX
CACGTGNNNNNSRGGAAARN	ERF:CLOCK
CACGTGNNNRGATTAN	ARNTL:PITX1
CAGCTGNNNNNNCCCGCAY	GCM1:FIGLA
CYAATAAAATGYN	TEAD4:HOXB13
GTMAACAGGAWRN	ETV5:FOX11
GTMAATAAGGGYRN	GCM1:FOXO1
TAATGNNNNNCGGAAGTN	HOXB2:ELK3
TAATKRCCGGAAGNN	HOXB2:ELK1
TAATKRNNNNGGAAGTN	HOXB2:ELF1
TAATKRNNNNGGAAGTN	HOXB2:ELF1
TAATTRNNNNCGGAARYN	ETV2:DLX3
TGACAKNNNAACAATGN	MEIS1:SOX2
TGACANNNTAATKR	MEIS1:DLX2
TGACASTAATKR	MEIS1:DRGX
TGTTGANGCGGN	GCM1:FOX11
TGTTGNCGGAWRN	ETV5:FOX11
TGTTKMCGGAWRN	ERF:FOX11
TGTTKMCGGAWRNN	FLI1:FOX11
TNRCACCGGAAGN	ELK1:EOMES
TNRCACCGGAAGNN	ELK1:TBX21
TNRCACCGGAWN	ETV5:EOMES
TTACGTNNNNNNNNNNCCCGGAANN	ETV2:TEF
ACCGGAARTNNNYAATTA	FLI1:DLX2
AGGTGNGAARGGATTA	PITX1:TBX21
AGGTGTGNNNNATCRAT	CUX1:TBX3
ANCCGGATNNNNNNMATTA	HOXB2:SPDEF
ARGTGNNNNNNNATAAA	HOXB13:TBX21
ATCRATNNNNNNNNNSYATTGTT	CUX1:SOX15
CACGTGNNNTAATKAT	CLOCK:EVX1
CASSTGNNNNNNNNNNNTGCGGG	GCM1:FIGLA
CASSTGNNNNNNNNNNNTGCGGG	GCM1:FIGLA
CASSTGNNNNNNNNNTGCGGG	GCM1:FIGLA
CASSTGNNNNNNNTGCGGG	GCM1:FIGLA
CYCATAAANNTGTCA	MEIS2:HOXA13

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GGAATKNNCASSTG	TEAD4:FIGLA
GGWATGNNNRTAAA	TEAD4:HOXA13
GGWATGNNNRTAAA	TEAD4:HOXB13
GTTGCYNNNNNNNNNNNNNCASSTG	RFX3:FIGLA
GWMAACANNSYMRTAAA	FOXO1:HOXB13
GWMAACAYMRTAAA	FOXO1:HOXB13
TAATCCNNNNCASSTG	PITX1:FIGLA
TAATKAGGTGNKA	HOXA3:EOMES
TAATKANNGGATTA	HOXB2:PITX1
TAATKRGGTGYKA	HOXB2:EOMES
TAATKRGGTGYKA	HOXB2:TBX21
TMACACCYAATA	CUX1:TBX21
TTTWATNRNMAACA	FOXJ2:HOXB13
AGGTGNTAATKWNNNNTN	MGA:EVX1
AGGTGTGNCSGTTR	MYBL1:TBX21
AGGTGTNNNGSGGGN	GCM2:TBX21
AGGTGTNRTRCGGGN	GCM1:TBX21
ANGTGTGAATWCY	TEAD4:TBX21
ANGTGSNNATAAAN	HOXB13:EOMES
ATRCGGGYNNNNNYWTTGTNN	GCM2:SOX15
CASSTGNNCCCGCAY	GCM1:FIGLA
CASSTGNNCCGGAWRYN	ETV2:TCF3
CASSTGNRNNNGGAAGNN	ETV5:TCF3
CTCRTAAAWNNNNRMCGTTR	MYBL1:HOXA13
GGATTANNARGTGTKN	PITX1:EOMES
GGATTANNNNATCRATN	CUX1:PITX1
GGWATGNNNNNNNCACGTGN	TEAD4:CLOCK
GGWATGNNNNNNNCACGTGN	TEAD4:CLOCK
GGWATGNRAGGTGNNR	TEAD4:EOMES
TAATKNNNNNGNNNNNNCTTCCNN	HOXB2:ETV7
TAATKRSCGGAWGN	ETV5:DRGX
TGACAGNWAATCRATR	MEIS1:ONECUT2
TGTTKATRCGGGN	GCM2:FOXI1
TGTTKMCGGAWRTN	ETV2:FOXI1
TMACACMGAARN	ETV2:TBX21
TTGCGYAANNSCGGAAGN	CEBPG:ELF1
ATCRATNNNNNYCRTAAA	CUX1:HOXA13
ATRAYANNNNCACGTG	CLOCK:EVX1
GGWATGYGTMAACA	TEAD4:FOXI1
TMACACYYMRTWAA	HOXC10:EOMES
TMRCACCTCRTWAA	HOXD12:EOMES
ARGTGNNANNNMWTAAAN	HOXB13:TBX21
ARGTGTKANNTTATNN	HOXB13:TBX21
ARGTGTKRNNNNNRGWATGY	TEAD4:TBX21
CYCRTAAATWCCN	TEAD4:HOXA13
GTMAACANNNNNNATCRATN	CUX1:FOXO1
TGMATATKCANNNNNTAATKR	POU2F1:DLX2
TGMATATKCANNNNNTAATKR	POU2F1:DLX2
TNSCCNNNGGSNNNNCACGTGN	TFAP2C:MAX
TNSCCNNNGGSNNNNCACGTGN	TFAP2C:MAX
TNSCCNNNGGSNNNNNNNNNNNNNNNCACGTGN	TFAP2C:MAX
TNSCCNNNGGSNNNNNNNNNNNNNNNCACGTGN	TFAP2C:MAX
TMRCACYTMATWAA	HOXC10:TBX21
ANGTGNNANNNNNNNNNNNNCNNMGGAWNN	ELK1:TBX21
ARGTGNNNAATATKYNNNCRCNN	POU2F1:EOMES
ATRSGGGNNNTTRCGYAAN	GCM1:CEBPB
GGWATGYNNNNNCRCGYGY	TEAD4:HES7
GGWATGYNNNNNNNCRCGYGY	TEAD4:HES7
TRGYAACNNNNCASSTGNN	RFX3:FIGLA
AGGYGYGANNNNNNNNNNNNNNTCRTWAA	HOXD12:EOMES
NNCGTAAAATTA	HOXB2:HOXB13

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NR MATATACCAATAAA		POU2F1:HOXB13
NT CGTAAAATGC		TEAD4:HOXB13
NT CGTAAATCA		PBX4:HOXA10
RCC GGAAGTAATTA		ELK1:HOXA1
YNN RATAATCAATCA		PBX4:HOXA10
NCC GATATGCAN		POU2F1:ELK1
NCC GATATGCAN		POU2F1:ETV1
NCC GATATGCAN		POU2F1:ETV4
NG ATGATGCAATNN		ATF4:CEBPD
NG CAGCTGCCGAWRYN		ETV2:NHLH1
NG CCACGCAAYN		CEBPG:CREB3L1
N MATGACACGCGCCMNN		E2F3:FOXO6
NM CCGGAACCGTTR		MYBL1:ELF1
NN CAGCTGCCGAWRYN		ERF:NHLH1
NNG AAAACCGAANM		FOXO1:ELF1
NN MATCACATAAAN		HOXB2:HOXB13
NN MCACGCGCCMNN		E2F3:FOXI1
NNN RATAATCACACNN		HOXB13:TBX3
NT GCCGGAAGTN		MEIS1:ELF1
NW AAACAGGAAGNN		FOXJ2:ELF1
RCC GGAATRSY		TEAD4:FLI1
RCC GATGTTKWN		ETV2:FOXO6
RCC GATGTTKWY		FLI1:FOXI1
RGA ATGCGGAAGTN		TEAD4:SPIB
RGA ATGCGGAAGTR		TEAD4:ELF1
RGA ATGCGGATN		TEAD4:SPDEF
RGG TGTTAATKN		HOXB2:EOMES
RM AGAAAACCGAANN		FOXJ2:ELF1
RN CGGATGTTKWN		ETV5:FOXO1
RN MTGATGCAATN		ATF4:TEF
RSC GGAAGTAATAAAN		HOXD12:ELK1
RSC GGAAGTAATAAAN		HOXD12:ELK3
RSC GGAAGTAATAAAN		HOXD12:ETV1
RSC GGAAGTAATAAAN		HOXD12:ETV4
RSC GGAAGTCGTAAAN		HOXD12:ELK3
RSC GGAAGTCGTAAAN		HOXD12:ETV4
RSC GATGTKKN		ELK1:FOXI1
RSC GATGTTGN		ETV5:FOXI1
RSC GATGTTKW		ETV2:FOXI1
RSC GATGTTKWN		ERF:FOXI1
RSC GTAAATKR		ELK1:HOXA3
RTG CGGGCGGAAGTN		GCM1:ELK1
RTG CGGGCGGAAGTN		GCM1:ELK3
RTG CGGGCGGAAGTR		GCM1:ETV4
RTG CGGGTAATAAAN		GCM1:HOXB13
RW MAACAGGAAGTN		FOXO1:ELF1
RW MAACAGGAARNN		FOXO1:ETV4
RW YAACAGGAAGYN		FOXO1:ELK1
SG CGCTAATTKN		E2F3:DRGX
WA ACAACACMY		FOXJ3:TBX21
W MSCGGATGTKNW		FOXO1:SPDEF
NC ACCTGNNNNNMATTA		HOXB2:TCF3
NC ACGTGNNGGATTA		PITX1:HES7
NC AGGTGNNNNNMATTA		HOXB2:TCF3
NCCC GCANNMATAAA		GCM1:HOXB13
NCCC GCANNMRTAAA		GCM1:HOXB13
NCC GGAAGNNNNNNYMATTA		ETV2:HOXA2
NCC GGAAGTMATTA		ETV2:HOXA2
NN ATCATNGTAAA		HOXB2:HOXB13
NN ATGAYGCAAT		CEBPG:ATF4
NN MAACAYNRTAAA		HOXB13:Fox

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NTAATCCNNWMAACA	FOXJ2:PITX1
NYNATMAATCA	PBX4:HOXA1
RCATTCCNNNNNNNCACGTG	TEAD4:MAX
RCATTCCNNNNNNNCACGTG	TEAD4:MAX
RCATTCCNNNNYAATTA	TEAD4:DLX3
RCATTCCNNNNYMATTA	TEAD4:DLX3
RCATTCCNNNNNNCATT	TEAD4:HOXA2
RCATTCCNNNNNNCATT	TEAD4:HOXA3
RCATTCCNNNNNTAATCC	TEAD4:PITX1
RCATTCCYNNNNCAATTA	TEAD4:DLX2
RCATTCCYNNNNCAATTA	TEAD4:DLX2
RCATTCCYNNNNNCATT	TEAD4:HOXA3
RCCGGAATRCC	TEAD4:ETV4
RCCGGAANNNNNNYAATTA	ETV2:DLX2
RCCGGAANNNNNNYAATTA	ETV2:DLX2
RGAATGCGGAWRT	TEAD4:SPDEF
RGAATGCNNRTAAA	TEAD4:HOXB13
RGAATGYGTGA	TEAD4:EOMES
RGAATGYNNACGTG	TEAD4:MAX
RGGTGNNNNNNNNNNYATTGT	SOX6:TBX21
RKRNGGGNNATAAA	GCM1:HOXA13
RNCGGAANNAACA	ETV2:FOX11
RRATGCARTAAA	TEAD4:HOXB13
RSCGGAATRCC	TEAD4:ERG
RSCGGAAGTMRTTA	HOXB2:ELK1
RSCGGAAGTMRTTA	HOXB2:ELK3
RSCGGAANNNNNGGATTA	ETV2:GSC2
RSCGGAANNNNNNGGATTA	ETV2:GSC2
RSCGGAANNNNNNNNYWTGT	ETV2:SOX15
RSCGGAANNNNNNNRTCGAT	FLI1:ONECUT2
RSCGGAANNNNNNYAATTA	ETV2:DLX3
RSCGGAANNNNNNYWTGT	ETV2:SOX15
RSCGGAANNNNNNYAATTA	ERF:DLX3
RSCGGAANNNNNNYAATTA	ETV2:DLX3
RSCGGAANNNNNNYAATTA	ETV5:DLX2
RSCGGAANNNNNNYMATTA	ERF:DLX2
RSCGGAANNNNYAATTA	ERF:DLX3
RSCGGAANYAATTA	ETV2:DRGX
RSCGGAANYNRTAAA	FLI1:HOXB13
RSCGGAARYAATTA	FLI1:DLX2
RSCGGAWNNNNNNNYMATTA	ERF:HOXA3
RSCGGAWRYATTA	ETV2:DRGX
SMGGAAGTMRTTA	HOXB2:ELF1
SYMRTAAANCTGTCA	MEIS1:HOXB13
SYNRTAAANNTGTCA	MEIS1:HOXA13
YAACGGNNNNNNNNNNNCACGTG	MYBL1:MAX
YAACGGNNNNNNNNNNNCACGTG	MYBL1:MAX
YRATTANNNNNNNNCACGTG	CLOCK:EVX1
NACAATRNNNNNGAATGY	TEAD4:SOX15
NACAATRNNNNNGAATGY	TEAD4:SOX15
NACTTCCGGYNNNNNGCAACSN	ETV2:RFX5
NAGGTGNTAATKR	HOXB2:TBX21
NCACGTGNNNNNCATATGN	CLOCK:BHLHA15
NCACGTGNNNNNSCGGAWRN	ETV5:CLOCK
NCAGCTGNNNNNCACGTGN	TFAP4:MAX
NCAGCTGNNNNNNNCACGTGN	TFAP4:MAX
NCCGGAAGTYRTAAAN	ETV2:HOXB13
NCCGGAANNCATATGN	FLI1:BHLHA15
NCCGGAANNNNNNNCAGCTGNN	ETV2:NHLH1
NCCGGAANYATAAAN	ETV2:HOXB13
NCCGGWNNNNNNNNNNNNSCATTAN	ELK1:HOXA3

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NGGTGTGNNGGCGCSN	E2F3:TBX21
NGGTGTGNNNGGCGCSN	E2F3:TBX21
NGGTGTNNNNNNNNNNNNNNNCCGGAWNNN	ETV2:EOMES
NMATGACACNGCGCCMNN	E2F3:FOXO6
NNACAATNNNNNNGAATGY	TEAD4:SOX6
NNARAAAACCGAAWMN	FOXJ3:ELF1
NNATGAYGCAAYN	ATF4:CEBPB
NNCACGTGACMGGAARNN	ERF:SREBF2
NNCACGTGNNNGCGGGYN	GCM1:MAX
NNCACGTGNNNNCCGGAANN	ERF:HES7
NNCACGTGNNNNCCGGAANN	ETV5:HES7
NNCACGTGNNNNNCCGGAANN	ERF:HES7
NNCACGTGNNNNNCCGGAWRY	ETV2:CLOCK
NNCACGTGNNNNNCCGGAWRY	ETV2:HES7
NNCACGTGNNNNNNNNNRTGCGGGYRN	GCM1:MAX
NNCAGCTGNNNNNNCACGTGNN	CLOCK:NHLH1
NNCAGCTGNNNNNNNATCGATN	CUX1:NHLH1
NNCAGCTGNNNNNNNTAATTN	TFAP4:DLX3
NNCAGCTGNNNNNTAATKR	TFAP4:DLX3
NNCAGGTGNNNNNMCGGAARYN	ETV2:FIGLA
NNCGGAANCAGGTGNN	ETV2:FIGLA
NNGATTANNNATGCAWNNN	POU2F1:GSC2
NNGTCACGCNNCATTAN	HOXB2:PAX5
NNGTCACGNNTCATTNN	HOXB2:PAX1
NNMGGAARNNRATAAN	ERF:HOXB13
NNNACGANNNNNNTCGTNNN	HOXD12:EOMES
NNNCGGGNNNGGTGTNN	GCM2:TBX21
NNNGMATAACAAWRRN	POU2F1:SOX15
NNNNGCGCSNNNNNCACGTGNN	E2F3:HES7
NNNRATAANCTGTN	HOXB13:MEIS1
NNNTTCCGSNNNNNGCAACNN	ETV2:RFX5
NNRNGGCGGAARTN	GCM2:ELK1
NNSGCGCSNNNNATCGAYN	E2F3:ONECUT2
NNSGCGCSNNNNATCGAYN	E2F3:ONECUT2
NNSMGACGGAYNTCCKSNN	ELK1:ETV7
NNYMATTANNNNNNGGAAGNN	HOXB2:ELF1
NRCAGCTGNNNNNCACGTGNN	CLOCK:NHLH1
NRCATTNNNNNTAATTRN	TEAD4:DLX2
NRCATTNNNNNYAATTN	TEAD4:DLX2
NRCCCRNNCGGAAGNN	GCM1:ELF1
NRSCGGAAGNNGTAAAN	HOXD12:ELK1
NSCGGAARNCACGTGNN	ERF:SREBF2
NSCGGAARNNNNNMATTAN	HOXB2:ETV7
NSCGGAARNNNNNMATTAN	HOXB2:ETV7
NSCGGAARNNNNNNTCACACNN	ETV7:TBX21
NSCGGAARNNNNNNTCACACNN	ETV7:TBX21
NSCGGAWNTTACGTAAN	ELK1:TEF
NSMGACGGAYNTCCKSN	ELK1:SPDEF
NSMGACGGAYNTCCKSN	ERF:ETV7
NSMGACGGAYNTCCKSN	ETV2:ETV7
NSMGACGGAYNTCCKSN	FLI1:ETV7
NTAATKRSNMRTAAAN	HOXD12:HOXA3
NTAATNRSNYMRTAAAN	HOXD12:HOXA3
NTGACRNNNNNNCACGTGN	MEIS1:MAX
NTGTTGATRNGGGN	GCM1:FOXJ1
NTGTTGNCGGAARNN	FOXO1:ELF1
NTTTAYNNCCGGAARNN	HOXD12:ELK3
NYMATTANNNNNACAATR	HOXB2:SOX15
NYMATTANNNNNCACGTGNN	HOXB2:NHLH1
NYMATTANNNNNACAATR	HOXB2:SOX15
NYMATTANNNNNNCAGCTGNN	HOXB2:NHLH1

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NYMATTANNNNNNNNCAGCTGNN	HOXB2:NHLH1
NYMATTANNNNNNNNGGAAGNN	HOXB2:ELF1
RAGGTSRNNNNNNNNNNNNNNNNCGGAAGYN	ELK1:TBX21
RCATTCCNNATCGAYN	TEAD4:ONECUT2
RCATTCCNNNNNTAATKR	TEAD4:DLX3
RCATTCCNNNNNNNNNNNNNNNGCAACN	TEAD4:RFX5
RCATTCCNNNNNNNNNNNNNNNGCAACN	TEAD4:RFX5
RCATTCCNNNNNTAATKR	TEAD4:DLX3
RCATTCCYNNNNCAAGGTCAN	TEAD4:ESRRB
RCATTCCYNNNNNTAATTR	TEAD4:DLX2
RCCGGAANNNNNATCGATN	ETV2:ONECUT2
RCCGGAANNNNNATCGATN	ETV2:ONECUT2
RCCGGAANNNNNNNNNNACACCTN	ETV2:EOMES
RCCGGAANNNNNNNNNNACACCTN	ETV2:TBX21
RCCGGAANNNNNNNNNNTAATCCN	ETV2:GSC2
RCCGGAANNNNNNNNTAATCCN	ETV2:GSC2
RCCGGAANNNNNNNNTAATCCN	ETV2:GSC2
RCCGGAWGTTKKN	FOXO1:ETV1
RCCGGAWGTTKKN	FOXO1:ETV4
RCCGGAWGTTKKW	FOXO1:ELK3
RGAATGCGGAARYNNNTCCN	TEAD4:ETV7
RGAATSCGGAAGYN	TEAD4:ELK1
RGGTGTNNNNNNNNNGAATGYN	TEAD4:EOMES
RGGTGYTAATWR	ALX4:TBX21
RGTGTNNNAATATKNN	POU2F1:EOMES
RGWATGTTAATCCS	TEAD4:GSC2
RKCACGTGNNNNMCATATGKN	ARNTL:BHLHA15
RKRNRGGCGGAARCGGAAGNN	GCM1:ELK1
RMATWCCGGAWRN	TEAD4:ELK1
RNCGGAANNNNNNNNNNGCAACN	ETV2:RFX5
RNCGGANNTTGCGCAAN	FLI1:CEBPB
RNCGGANNTTGCGCAAN	FLI1:CEBPD
RRGTGTNNNNNNNNNACAATRN	SOX6:TBX21
RSCGGAATRCM	TEAD4:ETV1
RSCGGAAGNNGTAAAN	ELK1:HOXB13
RSCGGAAGTNGTAAAN	HOXD12:ETV1
RSCGGAANCACGTGN	ERF:MAX
RSCGGAANCACGTGN	FLI1:MAX
RSCGGAANNNNNNCACGTGNN	ETV2:HES7
RSCGGAANNNNNNCACGTGNN	ETV2:HES7
RSCGGAANNNNNNTAATKR	ETV2:DLX3
RSCGGAANNNNNNRTCGATN	ERF:ONECUT2
RSCGGAANNNNNYAATTAN	FLI1:DLX2
RSCGGAANNRYMAACAN	ETV2:FOXI1
RSCGGAANRWMAACAN	ELK1:FOXI1
RSCGGAARCAGGTGN	ETV5:FIGLA
RSCGGAARYNNNTAAAN	HOXB13:ETV1
RSCGGANNCATATGK	ETV2:BHLHA15
RSCGGATGTTRTN	FOXO1:ELK1
RSCGGAWNNNNNNNACAATRN	ETV2:SOX15
RSCGGAWNNNNNNNACAATRN	ETV2:SOX15
RSCGGWAATKR	ETV5:EVX1
RSCGGWAATKR	ETV5:HOXA2
RS GTGNNNAACGK	MYBL1:EOMES
RTAAACAYNRTAAAN	FOXJ2:HOXB13
RTAAACMGGAARYN	ERF:FOXI1
RTAAACMGGAARYN	FLI1:FOXI1
RTAAATANGGGNN	GCM1:FOXI1
RTAAATANGGGNN	GCM2:FOXI1
RTCACGYSNCCGGAWN	ETV2:SREBF2
RTGCGGGNAGGTGNNN	GCM2:EOMES

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RTGCGGGNNATCGATR	GCM1:ONECUT2
RTGCGGGNNNNNNNCACGTGN	GCM1:MAX
RTGCGGGNNNTCGATR	GCM1:ONECUT2
RTMAACAGGAAGTN	FOXO1:ELK3
RTMAACAGGAAGTN	FOXO1:FLI1
RTMAACAGGAARN	ERF:FOXO1
RTMAACAGGAWRN	ETV5:FOXO1
RTRCGGGTAATAAAN	GCM2:HOXA13
RTRNNGGCGGAAGTN	GCM1:ETV1
RTRYGGGCGGAARKN	GCM1:ERG
RWCACGTGNCCGAANN	ELK1:SREBF2
RWMAACAGGNNNNNNNTTCNN	FOXO1:ETV7
SGCGCCNNNNNNNNNNNCAGCTGNN	E2F1:NHLH1
SGCGCNNNNNNCGGAAGN	E2F1:ELK1
SGCGCNNNNNNNNNNNCGGAAGN	E2F1:ELK1
SGCGCNNNNNNNNNNNCGGAAGN	E2F1:ELK1
SGTCACGCNTCATNN	HOXB2:PAX9
SYMATTANNNNNNRGCAACN	HOXB2:RFX5
WTGMATAACAATR	POU2F1:SOX15
YNATTANRGGTGTGAN	MGA:DLX3
NCACGTGNNNNNNNCATWCC	TEAD4:CLOCK
NCACGTGNNNNNNNCATWCC	TEAD4:CLOCK
NCAGCTGNNNNNNNNTRCGGG	GCM1:NHLH1
NCAGCTGNNNNNNNNTRCGGG	GCM1:NHLH1
NCCGGAANNNNNNMATWCC	TEAD4:ELK3
NCRCGTGNNNGGATTA	PITX1:HES7
NGTGNNNNMATATKNNNACACC	POU2F1:TBX21
NNRCGTGNNNGGATTA	PITX1:HES7
NNMATTAGTCACGCWTSRNTG	HOXB2:PAX1
NNMATTAGTCACGCWTSRNTG	HOXB2:PAX5
NNMATTAGTCACGCWTSRNTG	HOXB2:PAX9
NNNTATGCAGYGTKA	POU2F1:EOMES
NNTCCCGCNNCCNNNGGC	TFAP2C:E2F8
NSCNNNRGGCANNNNYMATTA	TFAP2C:DLX3
NSCGGACGGAWATCCGSNT	ETV2:SPDEF
NSCGGANNNNNGGMITTA	ERF:PITX1
NSCGGANNNNNGGMITTA	ERF:PITX1
NTRNGGGNNNCACGTG	GCM2:MAX
NTTTATNRNTMAACA	FOXJ2:HOXB13
RCATWCCNNNNNNNNYNNTAAA	TEAD4:HOXA13
RCATWCNNNNNGGATTA	TEAD4:PITX1
RCATWCNNNNNGGATTA	TEAD4:PITX1
RCATWCNNNNNNAGGTCA	TEAD4:ESRRB
RCATWCYNNNNNTAATCC	TEAD4:PITX1
RCATWCYNNNNNTAATCC	TEAD4:PITX1
RGGTGTKANNNNGGATTA	PITX1:EOMES
RMATATKCNNNNNNNNNNNNNNNNNRWMAACA	POU2F1:FOXO6
RMATATKCNNNNNNNNNNNNNNNNNRWMAACA	POU2F1:FOXO6
RMATATKCNNNNNNNNNNNNNNNNNRWMAACA	POU2F1:FOXO6
RNCGGAWGTMATTA	ETV5:HOXA2
RSCGGAANNNNNNNNNCATWCC	TEAD4:ERG
RSCGGAANTSRCGTGA	ELK1:SREBF2
RSCGGAASNGRTCATA	ELK1:ONECUT2
RSCGGAATKNNNNNNNNMATTA	ETV5:HOXA2
RSCGGAANNNNNYMATTA	ELK1:EVX1
RSMGGAWGYAATTA	ETV5:DRGX
RTAAACWNATWAAA	FOXJ2:HOXB13
RTGNKGGCGGAWG	GCM1:SPDEF
RTRCGGGNNNGATTA	GCM2:PITX1
RTRCGGGNNNNTTACGTAA	GCM2:TEF
RWMAACASYMRTWAAA	FOXO1:HOXB13

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SCC>NNNGGCNNYAATTA	TFAP2C:DRGX
SYAATTANWGGTGYGA	MGA:DLX2
WTWTGCATANNTAATTA	POU2F1:DLX2
YNRCACSTCGTWAA	HOXD12:TBX21
NAACGGNNATYGANN	MYBL1:ONECUT2
NATCGATNNNNNGCCTNNGGSNN	TFAP2C:ONECUT2
NATCGATNNNNNNNGCCTNNGGSNN	TFAP2C:ONECUT2
NATCGATNNNNNNNGCCTNNGGSNN	TFAP2C:ONECUT2
NATCRATNNNNNNNAACAATRS	CUX1:SOX15
NATCRATNNNNNNNAACAATRS	CUX1:SOX15
NATTTTCNNNACAATRN	POU2F1:SOX2
NCACGTGNNYAACSGNN	MYBL1:MAX
NCAGGTGNGWATGYN	TEAD4:TCF3
NCAGSTGNNNNNNNGTAATKR	TFAP4:DLX3
NCAGSTGNNNNNNNGTAATKR	TFAP4:DLX3
NCASSTGKNNNNNNNNCACGTGN	CLOCK:FIGLA
NCASSTGKNNNNNNNNCACGTGN	CLOCK:FIGLA
NCCGGAAGYNNCNTAGCAACS	ETV2:RFX5
NCCGGAANNNNNNCACGYGNN	ERF:HES7
NCCGGAANTNRTAAAN	FLI1:HOXB13
NCCGGANNCASSTGY	FLI1:TCF3
NGATAASNNNRGWATGY	TEAD4:GATA3
NGCCTNNGGSNNCGGAAGYN	TFAP2C:ELK1
NGGTGTGNNGGCGCSNNNNCRMN	E2F3:TBX21
NNCACGTGNNNNNRGMTTAN	ARNTL:PITX1
NNCAGCTGNNNNNNNNATYGATN	CUX1:NHLH1
NNCGGAWGYMATTAN	FLI1:DRGX
NNGGAMGGATKTCCGSN	ETV5:ETV7
NNGTMAATANGGGYR	GCM1:FOXJ1
NNGYGNNNNNNNNWAACAACACNN	FOXJ3:TBX21
NNGYGYSACATTCCN	TEAD4:EOMES
NNMGGAARTGCKGGN	GCM1:ELF1
NNMRTAAANTMACACNN	HOXB13:EOMES
NNNMGGAANNNNNTCCNNNRCAACN	RFX3:ETV7
NNNRTAAANNTNACACYN	HOXB13:TBX3
NNNRTAAAWATYGAYY	HOXB13:ONECUT2
NNRGYACNTCACGTGAY	RFX3:SREBF2
NRGTGTNANNNNNNNNNNNNCCGGAANN	ERF:TBX21
NRGYAACNNNCATATGKN	RFX3:BHLHA15
NRTCRATANCGGAARYN	ETV2:ONECUT2
NRTMAACMGGAARYN	ETV2:FOXJ1
NSCCNNNRGGCANNNNNNNTAATKR	TFAP2C:DLX3
NSCCNNNRGGCANNNNNNNTAATKR	TFAP2C:DLX3
NTCACACMNNNATCRATN	CUX1:TBX21
NTGACAGNTAATCRATAN	MEIS2:ONECUT2
NTNNNGGCGGAAGNNNTTCCNNN	GCM1:ETV7
NTRNGGGCGGAAGNNNTTCCNNN	GCM1:ETV7
NTRYGGGNNNTGGCGGARN	GCM2:E2F8
NYMRTAAANATYGATN	CUX1:HOXA13
RATTCCNNNNNNNNNNCASSTGN	TEAD4:FIGLA
RCATWCCNNNNNNNNNNNRWGCGTGACN	TEAD4:PAX5
RCATWCCNNNNNNNTTAYNNN	TEAD4:HOXA13
RCCGGAANNNNNNRATCRATN	ETV2:ONECUT2
RCCGGAARCASSTGNN	TFAP4:ETV4
RCCGGANRNNCGGWATKN	TEAD4:ELK1
RGGTGNTAAKCCN	MGA:PITX1
RGGTGNTAATNNNNNNNNNCASYNN	ALX4:TBX21
RGWATGYTAATKN	TEAD4:DRGX
RGWATGYTAATKR	TEAD4:DLX2
RNCGGAAGNNNNNCASSTGN	ETV5:TCF3
RNCGGAANYNRTWAAN	ELK1:HOXB13

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RNGTGNNNNNNNNNNNNNRRCRCGGAWSN	ETV5:EOMES
RRGTGTKNNNNNNNNNNNNNNCMGGANN	ERF:EOMES
RRTCRATNNNCGGAARYN	ETV2:ONECUT2
RSCGGAANCAGSTGNN	TFAP4:ETV1
RSCGGAANCASCTGNN	ERF:FIGLA
RSCGGAANCASSTGN	FLI1:FIGLA
RSCGGAANNNNNGGMITAN	ETV2:PITX1
RSCGGAASNGRTCGATAN	ELK1:ONECUT2
RSCGGANNTTGCGYAAN	ETV2:CEBPD
RSCGGAWRCASSTGN	TFAP4:FLI1
RSCGGWAATNNNNNATTAN	ETV5:EVX1
RSCGGWAATNNNNNATTAN	ETV5:EVX1
RSCGGWAATNNNYATTAN	ETV2:EVX1
RSMGGAANTTGCGYAAN	ERF:CEBPD
RSMGGAARTNNTAAAN	HOXB13:ELK1
RTAAACANNNNNNATCRATN	CUX1:FOXI1
RTAAACANNNNNNATCRATN	CUX1:FOXO6
RTMAACATRNGGGN	GCM1:FOXI1
RTMAATAMGGGYRN	GCM1:FOXO1
RTMRCGTGACGGAWGN	ETV2:SREBF2
RTRCGGGNNNNNNNYWTTGTNN	GCM2:SOX15
RTRCGGGNNNNNNNTAATKR	GCM2:DLX3
RTRCGGGNNNNNNNTAATKR	GCM2:DLX3
RTRCGGGNNNNNNNTAATTR	GCM2:DLX2
RTRCGGGNNNNRNACAAN	GCM2:SOX15
RTRCGGGNNNNRNACAAN	GCM2:SOX15
RTRCGGGSGATTAN	GCM2:PITX1
RTRNGGGTNNTAAAN	GCM1:HOXB13
RTRSGGGNAATTAN	GCM2:DRGX
RTRSGGGNAATTAN	GCM2:DRGX
RTRSGGGNNATTGTKY	GCM1:SOX2
RTRSGGGNTAATKR	GCM1:HOXA2
RTRSKGGCGGANNNNNNATCCNN	GCM1:SPDEF
RTRSKGGCGGANNNNNNATCCNN	GCM1:SPDEF
SYMRTAAANATYGATN	CUX1:HOXB13
SYMRTAAANNNNCASSTGN	HOXD12:FIGLA
WGTTKMCGGAWRTN	ELK1:FOXI1
YNATTAGTCACGCWTSRNR	HOXA3:PAX5
NCASSTGNNNNNNNNNRTRCGGG	GCM2:FIGLA
NGGTGTGNNNGGCGSNNNCRC	E2F1:EOMES
NNCGGANGNNNTWAA	ETV5:HOXB13
NNCGGAWGTNRTWAA	ETV5:HOXA13
NTATKCAGYGTNA	POU2F1:EOMES
NTATKCAGYGTNA	POU2F1:TBX21
RATCRATNNNNNNNNNNNRGYAAC	CUX1:RFX5
RATCRATNNNNNNNNNNNRGYAAC	CUX1:RFX5
RCATWCCNNNNNNNNNNNNNNRTMAACA	TEAD4:FOXI1
RCATWCCNNNNNNNNNNNNNNRTMAACA	TEAD4:FOXI1
RCATWCCNNNNNNNNNNNNNNRTMAACA	TEAD4:FOXI1
RCATWCCNNNNNNNNNNNNNNRTMAACA	TEAD4:FOXI1
RCATWCYNNNNNTAATYRNATTA	TEAD4:ALX4
RSCGGAACYACGCWYSANTG	ELK1:PAX1
RSCGGAACYACGCWYSANTG	ELK1:PAX5
RSCGGAACYACGCWYSANTG	ELK1:PAX9
RSCGGAANNAGGYGYNA	ELK1:EOMES
RSCGGAANNRTMAAYA	ELK1:FOXI1
RSCGGAANNRTMAAYA	ELK1:FOXI1
RTRCGGGNNCASSTG	GCM2:FIGLA
RWMAACAYCRTWAA	FOXO1:HOXA10
SCCNNNNGGCATNGTWAA	TFAP2C:HOXB13
NATYGATNNNNNNNNNGCCTNNGGSNN	TFAP2C:ONECUT2

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NCASSTGNNNNNNNNCSGTTR	MYBL1:FIGLA
NCASSTGNNNNNNNNCSGTTR	MYBL1:FIGLA
NCASSTGNNNNNNNNCSGTTR	MYBL1:FIGLA
NCASSTGNNNNNNYAACSGYN	MYBL1:FIGLA
NCASSTGNNNNNNYAACSGYN	MYBL1:FIGLA
NCCATAYWNGGWNNNNNATCRATN	CUX1:SRF
NGGTGTGNNNNNTMATTWGCRRN	HOXB2:TBX21
NRGCAACNNNNCRGCGYNN	RFX3:HES7
NRGYAACNNNNNCCTWWWNNNGGN	RFX3:SRF
NSCGGANNTTRCGYAAN	ETV5:CEBPD
NTTRCGYAANNNNNNGGAATGY	TEAD4:CEBPB
RCATTCCNNCRGCGYNN	TEAD4:HES7
RCATTCCNNNNNNCRGCGYNN	TEAD4:HES7
RGGTGNGANNNNNNNNTNNCACCGGAAGY	ELK1:EOMES
RGGTGTNNNGGCGSNNTNNCRSNN	E2F1:EOMES
RGTGTKRNNNNNNNNNNCNCMGGGAARN	ETV2:TBX21
RGWATSCGGATGNNNNNTCCKNNN	TEAD4:SPDEF
RRCRCGYGNNNNNSGCGCSN	E2F1:HES7
RSCGGAANNRTMAAYAN	ELK1:FOXJ1
RSCGGAWNTTRCGYAAN	ETV2:TEF
RTGNKGGCGGAWGNNNNNTCCGGNN	GCM1:SPDEF
RTGNKGGCGGAWGNNNNNTCCGGNN	GCM1:SPDEF
RTRCGGGNNNNNNNATCRATN	GCM2:ONECUT2
RTRNKGGTNNNGCACGYGNN	GCM2:HES7
SCCANNNGGSNNNGGAAGNNNTTCCNN	TFAP2C:ETV7
SRCRCGYGSYNNNNNSGCGCSN	E2F1:HES7
WNGTGYKAMATWCY	TEAD4:EOMES
MTRSGGGNNNNNNNTTRCGYAAN	GCM1:CEBPB
MTRSGGGNNNNNNNTTRCGYAAN	GCM1:CEBPB
NGGTGTNNNNNNNNNNNNNCACNTNNTWAN	HOXD12:TBX21
NGYGYTAAAYNNNNNNNTNACACNN	ALX4:EOMES
NNCRCGYGNNNNNNNSCCNNNGGS	TFAP2C:HES7
NNCRCGYGNNNNNNNSCCNNNGGS	TFAP2C:HES7
NNGYGNNNNNGGCGSNNNNCRCNN	E2F3:EOMES
NNNTATGCNNNGYGANNNNNNNNNNNNCRCN	POU2F1:EOMES
NNSYGNCNAACNNNNNNNCACRCNN	MYBL1:EOMES
NTTRCGYAANNNNNNNNRGWATGY	TEAD4:CEBPD
NTTRCGYAANNNNNNNNRGWATGY	TEAD4:CEBPD
NTTRCGYAANNNNNNNNRGWATGY	TEAD4:CEBPD
RGGTGWKNNNNNNNNNTNNCRTRNGGGN	GCM1:TBX21
RGWATGYNNNTTRCGYAAN	TEAD4:CEBPD
NGYGNNAMATWCYNNNTMRCRCN	TEAD4:EOMES

Table 15. Gene Complex Motif Patterns

Pattern	Number of each pattern in Gene Complex Table
AB	11
ABA	27
ABAB	29
ABABA	25
ABABAB	23
ABABABA	5
ABABABAB	11
ABABABABA	1
ABABABABAB	6
ABABABABABA	1

Table 16. Gene Complex Motif Patterns

Pattern	Number of each pattern in Gene Complex Table
BA	6
BAB	46
BABA	58
BABAB	155
BABABA	45
BABABAB	95
BABABABA	22
BABABABAB	28
BABABABABAB	14
BABABABABABABAB	1

Table 17. Single Gene Motif Patterns

Pattern	Number of each pattern in Single Gene Table
AB	42
ABA	97
ABAB	37
ABABA	40
ABABAB	4
ABABABA	28
ABABABAB	4
ABABABABA	4
ABABABABABA	1

Table 18. Single Gene Motif Patterns

Pattern	Number of each pattern in Single Gene Table
BA	34
BAB	186
BABA	40
BABAB	120
BABABA	15
BABABAB	47
BABABABA	8
BABABABAB	8
BABABABABA	1
BABABABABAB	2

Table 19. IUPAC Nucoetide Code

IUPAC code	Base
A	Adenine
C	Cytosine
G	Guanine
T (or U)	Thymine (or Uracil)
R	A or G
Y	C or T
S	G or C
W	A or T
K	G or T
M	A or C
B	C or G or T

D	A or G or T
H	A or C or T
V	A or C or G
N	Any base
“-” or “-”	gap

Table 20. Mutations in the OTC gene associated with OTC deficiency

No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
1			c.-366A>G		Regulatory	
2	Exon 1	1	c.1A>G	p.Met1Val	Missense	
3		1	c.1A>T	p.Met1Leu	Missense	
4		1	c.2T>C	p.Met1Thr	Missense	
5		1	c.3G>A	p.Met1Ile	Missense	
6		9	c.25T>G	p.Leu9*	Nonsense	
7		10,11	c.28_31del AACA	p.Asn10_Asn11	Frameshift	
8		10	c.29dupA	p.fsX	Frameshift	
9		14	c.40delT	p.Phe14Leufs	Frameshift	
10		18	c.53delA	p.His18Profs	Frameshift	
11		23	c.67C>T	p.Arg23*	Nonsense	
12		26	c.77G>A	p.Arg26Gln	Missense	0%
13		26	c.77G>C	p.Arg26Pro	Missense	
14	Intron 1		c.77+1G>T		Splice site error	
15			c.77+1G>A		Splice site error	
16			c.77+2dupT		Splice site error	
17			c.77+3_6del AAGT		Splice site error	
18			c.77+4A>C		Splice site error	0%
19			c.77+5G>A		Splice site error	
20			c.78-3C>G		Splice site error	<5%
21			c.78-1G>C		Splice site error	
22	Exon 2	32	c.94C>T	p.Gln32*	Nonsense	
23		36	c.106C>T	p.Gln36*	Nonsense	
24		39	c.115G>T	p.Gly39Cys	Missense	
25		39	c.116G>A	p.Gly39Asp	Missense	
26		40	c.118C>T	p.Arg40Cys	Missense	
27		40	c.119G>A	p.Arg40His	Missense	6%
28		40	c.119G>T	p.Arg40Leu	Missense	
29		41	c.122A>G	p.Asp41Gly	Missense	
30		43	c.126_128del TCT	p.Leu43del	In frame indel	
31		43	c.127C>T	p.Leu43Phe	Missense	
32		44	c.131C>T	p.Thr44Ile	Missense	
33		45	c.133C>G	p.Leu45Val	Missense	
34		45	c.134T>C	p.Leu45Pro	Missense	
35		45	c.135dupA	p.fsX	Frameshift	
36		45-47	c.135delA	p.Asn47Thrfs	Frameshift	0%
37		47	c.140_141ins G	p.Asn47delinsLysLeufs	Frameshift	

¹ Nucleotide +1 is the A of the translation initiation codon of the NM_000531.3.² For deletions or insertions, the cDNA nucleotide number is given starting with the A of the translation initiation codon.³ %) residual activity in liver or intestine or determined by expression studies; [15N] residual nitrogen incorporation into urea. %) residual activity in liver or intestine or determined by expression studies; [15N] residual nitrogen incorporation into urea.

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
38		47	c.140A>T	p.Asn47Ile	Missense	
39		47	c.140A>C	p.Asn47Thr	Missense	
40		48	c.143T>C	p.Phe48Ser	Missense	
41		49	c.145A>C	p.Thr49Pro	Missense	
42		50	c.148G>A	p.Gly50Arg	Missense	
43		50	c.148G>T	p.Gly50*	Nonsense	
44		52	c.154G>A	p.Glu52Lys	Missense	
45		52	c.154G>T	p.Glu52*	Nonsense	
46		52	c.155A>G	p.Glu52Gly	Missense	
47		52	c.156A>T	p.Glu52Asp	Missense	4%
48		53	c.158T>C	p.Ile53Thr	Missense	
49		53	c.158T>G	p.Ile53Ser	Missense	
50		55	c.163T>G	p.Tyr55Asp	Missense	28%
51		56	c.167T>C	p.Met56Thr	Missense	[54%]
52		57	c.170T>A	p.Leu57Gln	Missense	
53		58	c.174G>A	p.Trp58*	Nonsense	
54		59	c.176T>G	Leu59Arg	Missense	
55		60	c.179C>T	p.Ser60Leu	Missense	
56		62	c.184G>C	p.Asp62His	Missense	
57		62	c.185A>G	p.Asp62Gly	Missense	
58		63	c.188T>C	p.Leu63Pro	Missense	
59		67	c.200T>G	p.Ile67Arg	Missense	
60		69	c.205C>T	p.Gln69*	Nonsense	
61	Intron 2		c.216+1G>A		Splice site error	
62			c.216+1G>T		Splice site error	10%
63			c.217-1G>A		Splice site error	
64	Exon 3	73	c.219T>G	p.Tyr73*	Nonsense	
65		76	c.227T>C	p.Leu76Ser	Missense	
66		77	c.231G>T	p.Leu77Phe	Missense	[35%]
67		78	c.232C>T	p.Gln78*	Nonsense	
68		79	c.236G>A	p.Gly79Glu	Missense	0%
69		80	c.238A>G	p.Lys80Glu	Missense	
70		80	c.240G>T	p.Lys80Asn	Missense	
71		81-82	c.243_245del CTT	p.Leu244del	In frame indel	
72		82	c.245T>G	p.Leu82*	Nonsense	
73		82	c.245T>A	p.Leu82*	Nonsense	
74		83	c.248G>A	p.Gly83Asp	Missense	
75		85	c.256dupT	p.fsX	Frameshift	
76		88	c.264A>T	p.Lys88Asn	Missense	3%
77		90	c.268A>G	p.Ser90Gly	Missense	(<20%)
78		90	c.269G>A	p.Ser90Asn	Missense	
79		90	c.270T>G	p.Ser90Arg	Missense	
80		91	c.271delA	p.Thr91Leufs	Frameshift	
81		92	c.274C>G	p.Arg92Gly	Missense	
82		92	c.274C>T	p.Arg92*	Nonsense	0%
83		92	c.275G>T	p.Arg92Leu	Missense	
84		92	c.275G>A	p.Arg92Gln	Missense	0%
85		92	c.275G>C	p.Arg92Pro	Missense	
86		93	c.277A>G	p.Thr93Ala	Missense	
87		94	c.281G>C	p.Arg94Thr	Missense	
88		95	c.284T>C	p.Leu95Ser	Missense	
89		98	c.292G>A	p.Glu98Lys	Missense	33%
90	Intron 3		c.298+1G>A		Splice site error	
91			c.298+1G>T		Splice site error	
92			c.298+1_5del GTAAG		Splice site error	

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
93			c.298+5G>C		Splice site error	
94			c.299-8T>A		Splice site error	
95			c.299-7A>G		Splice site error	
96	Exon 4	100	c.299G>A	p.Gly100Asp	Missense	
97		100	c.298G>C	Gly100Arg	Missense	
98		102	c.304G>C	p.Ala102Pro	Missense	
99		102	c.305C>A	p.Ala102Glu	Missense	
100		105	c.314G>T	p.Gly105Val	Missense	
101		105	c.314G>A	p.Gly105Glu	Missense	
102		106	c.316G>A	p.Gly106Arg	Missense	
103		106	c.317G>A	p.Gly106Glu	Missense	<20%
104		106	c.317G>T	p.Gly106Val	Missense	
105		109	c.327T>C	p.Cys109Arg	Missense	
106		109-110	c.327delT	p.Cys109Cysfsx	Frameshift	
107			c.341-342delAA	p.fsX	Frameshift	
108		111	c.332T>C	p.Leu111Pro	Missense	
109		117	c.350A>G	p.His117Arg	Missense	18%
110		117	c.350A>T	p.His117Leu	Missense	
111		120	c.358_359delGT	p.Val120Glu fs	Frameshift	
112		122	c.364_365insTT	p.Glu122delinsValLys fs	Frameshift	
113		125	c.374C>T	p.Thr125Met	Missense	<1%
114		125	c.375delG	p.Thr125Thrfs	Frameshift	
115		126	c.377A>G	p.Asp126Gly	Missense	
116		129	c.386G>C	p.Arg129Pro	Splice site error	
117		129	c.386G>A	p.Arg129His	Splice site error	3.50%
118		129	c.386G>T	p.Arg129Leu	Splice site error	
119		129	c.385C>T	p.Arg129Cys	Splice site error	
120	Intron 4		c.386+1G>T		Splice site error	
121			c.386+1G>A		Splice site error	
122			c.386+1G>C		Splice site error	
123			c.386+2T>C		Splice site error	
124			c.386+5G>A		Splice site error	
125			c.387-2A>T		Splice site error	
126			c.387-2A>C		Splice site error	
127			c.387-2A>G		Splice site error	
128	Exon 5	130-131	c.390_391insTTA	p.Val130_Leu131insLeu	In frame indel	
129		131	c.392T>C	p.Leu131Ser	Missense	
130		132	c.394T>C	p.Ser132Pro	Missense	
131		132	c.395C>T	p.Ser132Phe	Missense	[78.6%]
132		135	c.403delG	p.Ala135Glnfs	Frameshift	
133		135	c.404C>A	p.Ala135Glu	Missense	
134		136	c.407A>T	p.Asp136Val	Missense	
135		137	c.409G>C	p.Ala137Pro	Missense	
136		137	c.409G>A	p.Ala137Thr	Missense	
137		139	c.416T>C	p.Leu139Ser	Missense	
138		140	c.418G>C	p.Ala140Pro	Missense	
139		140	c.419C>A	p.Ala140Asp	Missense	
140		141	c.421C>G	p.Arg141Gly	Missense	
141		141	c.421C>T	p.Arg141*	Nonsense	
142		141	c.422G>A	p.Arg141Gln	Missense	0%
143		141	c.422G>C	p.Arg141Pro	Missense	
144		142	c.425T>A	p.Val142Glu	Missense	
145		143	c.429T>A	p.Tyr143*	Nonsense	

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
146		144	c.430A>T	p.Lys144*	Nonsense	
147		146	c.437C>G	p.Ser146*	Nonsense	
148		148	c.443T>C	p.Leu148Ser	Missense	
149		148	c.443T>G	p.Leu148Trp	Missense	
150		148	c.444G>C	p.Leu148Phe	Missense	
151		148	c.444G>T	p.Leu148Phe	Missense	17%
152		150-151	c.449delC	p.fsX	Frameshift	
153		151	c.452T>G	p.Leu151Arg	Missense	
154		152	c.455C>T	p.Ala152Val	Missense	3.70%
155		154	c.460G>T	p.Glu154*	Nonsense	0%
156		154	c.461_471del	p.Glu154Alafs*18	Frameshift	
157		155	c.463G>T	p.Ala155Ser	Missense	
158		155	c.463G>C	p.Ala155Pro	Missense	
159		155	c.464C>A	p.Ala155Glu	Missense	
160		158	c.472C>T	p.Pro158Ser	Missense	
161		159	c.476T>C	p.Ile159Thr	Missense	1.50%
162		159	c.477T>G	p.Ile159Met	Missense	
163		160	c.479T>G	p.Ile160Ser	Missense	
164		160	c.479T>A	p.Ile160Asn	Missense	
165		160	c.479T>C	p.Ile160Thr	Missense	
166		161	c.481A>G	p.Asn161Asp	Missense	
167		161	c.482A>G	p.Asn161Ser	Missense	
168		161	c.483T>A	p.Asn161Lys	Missense	<10%
169		161	c.483T>G	p.Asn161Lys	Missense	<10%
170		162	c.484G>C	p.Gly162Arg	Missense	
171		162	c.484G>A	p.Gly162Arg	Missense	
172		162	c.485G>A	p.Gly162Glu	Missense	
173		164	c.490T>C	p.Ser164Pro	Missense	
174		164	c.491C>G	p.Ser164*	Nonsense	
175		165	c.493G>T	p.Asp165Tyr	Missense	
176		167	c.501C>A	p.Tyr167*	Nonsense	0%
177		167	c.501C>G	p.Tyr167*	Nonsense	
178		168	c.503A>C	p.His168Pro	Missense	
179		168	c.503A>G	p.His168Arg	Missense	
180		168	c.504T>A	p.His168Gln	Missense	[69%]
181		169	c.505C>G	p.Pro169Ala	Missense	
182		169	c.506C>T	p.Pro169Leu	Missense	
183		169	c.506C>A	p.Pro169His	Missense	
184		172	c.514A>T	p.Ile172Phe	Missense	
185		172	c.516C>G	p.Ile172Met	Missense	
186		172	c.515T>A	Ile172Asn	Missense	
187		174	c.520G>C	p.Ala174Pro	Missense	
188		175	c.524A>G	p.Asp175Gly	Missense	
189		175	c.524A>T	p.Asp175Val	Missense	
190		176	c.526T>C	p.Tyr176His	Missense	
191		176	c.527A>G	p.Tyr176Cys	Missense	19%
192		176	c.527A>C	p.Tyr176Leu	Missense	
193		178	c.533C>T	p.Thr178Met	Missense	
194		177-178	c.530_533 dupTCAC	p.fsX	Frameshift	
195		178-179	c.532_537del ACGCTC	p.Thr178_Leu179del	In frame indel	
196		179	c.535C>T	p.Leu179Phe	Missense	
197		179	c.536T>C	p.Leu179Pro	Missense	
198		180	c.538C>T	p.Gln180*	Nonsense	
199		180	c.[539A>C (+) 540G>C]	p.Gln180Pro	Missense	<10%

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
200	Intron 5	180	c.540G>C	p.Gln180His	Splice site error	7.1% [43%]
201			c.540+1G>C		Splice site error	
202			c.540+2T>C		Splice site error	0%
203			c.540+2T>A		Splice site error	
204			c.541-2A>G		Splice site error	
205			c.540+265 G>A		Splice site error	
206	Exon 6		c540+2T>G		Splice site error	
207		181	c.542A>G	p.Glu181Gly	Missense	
208		182	c.545A>T	p.His182Leu	Missense	
209		183	c.547T>G	p.Tyr183Asp	Missense	
210		183	c.548A>G	p.Tyr183Cys	Missense	
211		186	c.557T>C	p.Leu186Pro	Missense	
212		188	c.561delA	p.Gln188fs	Frameshift	
213		188	c.562G>C	p.Gly188Arg	Missense	2%
214		188	c.562_563del GG	p.Gly188SfsX36	Frameshift	
215		188	c.563G>T	p.Gly188Val	Missense	
216		188	c.563G>C	p.Gly188Ala	Missense	
217		190	c.568delA	p.T190PfsX16	Frameshift	
218		191	c.571C>T	p.Leu191Phe	Missense	5.70%
219		191	c.571delC	p.Leu191SerfsX15	Frameshift	
220		191	c.572T>G	p.Leu191Arg	Missense	
221		192	c.576C>G	p.Ser192Arg	Missense	
222		193	c.577T>C	p.Trp193Arg	Missense	
223		193	c.577T>G	p.Trp193Gly	Missense	
224		193	c.578G>A	p.Trp193*	Nonsense	
225		193	c.579G>C	p.Trp193Cys	Missense	
226		194	c.581T>C	p.Ile194Thr	Missense	
227		195	c.583G>A	p.Gly195Arg	Missense	0%
228		195	c.583delG	p.Asp196Metfs	Frameshift	
229		196	c.586G>A	p.Asp196Asn	Missense	
230		196	c.586G>T	p.Asp196Tyr	Missense	
231		196	c.586G>C	p.Asp196His	Missense	
232		196	c.587A>T	p.Asp196Val	Missense	7%
233		197	c.589G>A	p.Gly197Arg	Missense	
234		197	c.590G>A	p.Gly197Glu	Missense	
235		197	c.589G>T	p.Gly197Trp	Missense	
236		198	c.593A>T	p.Asn198Ile	Missense	
237		198	c.594C>A	p.Asn198Lys	Missense	
238		199	c.595A>G	p.Asn199Asp	Missense	
239		199	c.596A>G	p.Asn199Ser	Missense	
240		199-200	c.(597_598) delTA	p.fsX	Frameshift	
241		201	c.602T>C	p.Leu201Pro	Missense	
242		202	c.604C>T	p.His202Tyr	Missense	[49%]
243		202	c.605A>C	p.His202Pro	Missense	
244		203	c.608C>G	p.Ser203Cys	Missense	
245		205	c.613A>G	p.Met205Val	Missense	
246		205	c.614T>C	p.Met205Thr	Missense	
247		206	c.617T>G	p.Met206Arg	Missense	
248		206	c.618G>C	p.Met206Ile	Missense	
249		207	c.620G>A	p.Ser207Asn	Missense	
250		207	c.621C>A	p.Ser207Arg	Missense	
251		208	c.622G>A	p.Ala208Thr	Missense	4%
252		209	c.626C>T	p.Ala209Val	Missense	1% [1.4%]
253		210	c.628A>C	p.Lys210Glu	Missense	

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
254		210	c.630A>C	p.Lys210Asn	Missense	
255		210	c.628A>C	p.Lys210Gln	Missense	0%
256		213	c.637T>A	p.Met213Lys	Missense	
257		213	c.637T>C	p.Met213Thr	Missense	
258		213	c.637T>G	p.Met213Arg	Missense	
259		214	c.640C>T	p.His214Tyr	Missense	
260		215	c.643C>T	p.Leu215Phe	Missense	17%
261		215-216	c.645_646ins T	p.Gln216delinsSerGly fs	Frameshift	
262		216	c.646C>G	p.Gln216Glu	Missense	
263		217	c.650C>A	p.Ala217Glu	Missense	
264		218	c.653C>T	p.Ala218Val	Missense	
265		220	c.658C>G	p.Pro220Ala	Missense	35%
266		220	c.659C>T	p.Pro220Leu	Missense	
267		221	c.663G>C	p.Lys221Asn	Missense	
268		221	c.663G>A	p.Lys221Lys	Splice site error	8%
269	Intron 6		c.663+1G>A		Splice site error	
270			c.663+1G>T		Splice site error	
271			c.663+1delG		Splice site error	
272			c.663+2T>C		Splice site error	
273			c.663+2dupT		Splice site error	
274			c.664-1G>A		Splice site error	
275			c.664-1delG	p.fsX	Frameshift	
276	Exon 7	222	c.664-667delinsAC	p.Gly222Thrfs*2	Frameshift	
277		224	c.670G>T	p.Glu224*	Nonsense	
278		225	c.673C>A	p.Pro225Thr	Missense	[42%]
279		225	c.674C>G	p.Pro225Arg	Missense	0%
280		225	c.674C>T	p.Pro225Leu	Missense	0% [0.45%]
281		233	c.698C>T	p.Ala233Val	Missense	
282		234	c.700G>T	p.Glu234*	Nonsense	
283		239	c.716A>T	p.Glu239Val	Missense	
284		239	c.716A>G	p.Glu239Gly	Missense	
285		239	c.717G>C	p.Glu239Asp	Missense	
286		239	c.717G>A	p.Glu239Glu	Splice site error	
287	Intron 7		c.717+1G>T		Splice site error	
288			c.717+1G>A		Splice site error	
289			c.717+2T>C		Splice site error	
290			c.717+3A>G		Splice site error	
291			c.717+7_22 delTCTTTA CATGTAAA GC		Splice site error	0-1.5%
292			c.718-2A>G		Splice site error	
293	Exon 8		c.718-4_729delCT AGAATGGT ACCAAG		Splice site error	
294		242	c.725C>T	p.Thr242Ile	Missense	
295		244	c.731T>A	p.Leu244Gln	Missense	8%
296		247	c.740C>A	p.Thr247Lys	Missense	0%
297		244-247	c.731_739del TGTGCTG A		In frame indel	
298		249	c.746A>G	p.Asp249Gly	Missense	
299		250	c.749C>T	p.Pro250Leu	Missense	
300		253	c.757G>A	p.Ala253Thr	Missense	
301		253	c.757G>C	p.Ala253Pro	Missense	
302		253	c.759delA	p.fsX	Frameshift	

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
303		254	c.760A>T	p.Ala254*	Nonsense	
304		255	c.764A>C	p.His255Pro	Missense	
305		260	c.779T>C	p.Leu260Ser	Missense	
306		262	c.784_792dup9	p.thr262_Thr264dup TDT	In frame indel	
307		262	c.785C>A	p.Thr262Lys	Missense	26%
308		262	c.785C>T	p.Thr262Ile	Missense	
309		263	c.787G>A	p.Asp263Asn	Missense	
310		263	c.788A>G	p.Asp263Gly	Missense	
311		264	c.790A>G	p.Thr264Ala	Missense	22%
312		264	c.791C>A	p.Thr264Asn	Missense	
313		264	c.791C>T	p.Thr264Ile	Missense	
314		265	c.793T>C	p.Trp265Arg	Missense	
315		265	c.794G>T	p.Trp265Leu	Missense	56%
316		265	c.795G>A	p.Trp265*	Nonsense	
317		265-268	c.796_805del	p.Ile265_Gly268delins AspfsX19	Frameshift	
318		267	c.799A>C	p.Ser267Arg	Missense	
319		268	c.803T>C	p.Met268Thr	Missense	6.70%
320		268	c.802A>G	p.Met268Val	Missense	
321		269	c.806G>A	p.Gly269Glu	Missense	2%
322		270	c.808C>T	p.Gln270*	Nonsense	
323		270	c.809A>C	p.Gln270Pro	Missense	
324		271-272	c.810_811del AGinsC	p.fsX	Frameshift	
325		272-273	c.817_819del GAG	p.Glu273del	In frame indel	5%
326		273	c.818delA	p.Glu273Glyfs	Frameshift	
327		277	c.829C>T	p.Arg277Trp	Missense	5% [59%]
328		277	c.830G>A	p.Arg277Gln	Missense	7%
329		277	c.830G>T	p.Arg277Leu	Missense	
330		279	c.835C>T	p.Gln279*	Nonsense	
331		281	c.842T>C	p.Phe281Ser	Missense	
332		284	c.853delC	p.Phe284fsX38	Frameshift	
333		285	c.853C>T	p.Gln285*	Nonsense	
334		287	c.860A>C	p.Thr287Pro	Missense	
335		287	c.861insAC	p.fsX	Frameshift	
336		286-289	c.867G>A r.856_867del GTTACAAT GAG	p.Val286_Lys289del	In frame indel	
337		289	c.867G>C	p.Lys289Asp	Missense	
338		289	c.867G>T	p.Lys289Asn	Missense	0%
339	Intron 8		c.867+1G>A		Splice site error	
340			c.867+1G>T		Splice site error	
341			c.867+1126A>G		Splice site error	
342			c.868-3T>C		Splice site error	
343	Exon 9	292	c.875delA	p.Val293Leufs	Frameshift	
344		294	c.882delT	p.Ala295Profs	Frameshift	
345		297	c.889G>T	p.Aso297Tyr	Missense	
346		297-298	c.889_892del GACT	p.fsX	Frameshift	0%
347		298	c.892_893del TG	p.Trp298Aspfs	Frameshift	
348		298	c.892T>C	p.Trip298Arg	Missense	
349		298	c.893G>C	p.Trp298Ser	Missense	0%

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
350		301	c.902T>C	p.Leu301Ser	Missense	
351		301	c.903A>T	p.Leu301Phe	Missense	3%
352		302	c.904C>T	p.His302Tyr	Missense	0%
353		302	c.905A>G	p.His302Arg	Missense	
354		302	c.905A>T	p.His302Leu	Missense	
355		302	c.906C>G	p.His302Gln	Missense	
356		302	c.906delC	p.Cys303Alafs	Frameshift	
357		303	c.907T>C	p.Cys303Arg	Missense	
358		303	c.907T>G	p.Cys303Gly	Missense	
359		303	c.908G>A	p.Cys303Tyr	Missense	
360		304	c.912G>T	p.Leu304Phe	Missense	6% [74%]
361		305	c.914C>G	p.Pro305Arg	Missense	
362		305	c.914C>A	p.Pro305His	Missense	
363		306	c.916A>T	p.Arg306*	Nonsense	
364		306	c.917G>C	p.Arg306Thr	Missense	
365		309-310	c.(925-927)delGAA	p.Glu309del	In frame indel	
366		310	c.928G>T	p.Glu310*	Nonsense	
367		310	c.929_931del AAG	p.Glu310del	In frame indel	
368		310	c.929A>G	p.Glu310Gly	Missense	
369		311	c.931G>A	p.Val311Met	Missense	
370		311	c.932T>A	p.Val311Glu	Missense	
371		314	c.940_942del GAA	p.Glu314del	In frame indel	
372		315	c.943G>T	p.Val315Phe	Missense	
373		315	c.944T>A	p.Val315Asp	Missense	
374		315	c.944T>G	p.Val315Gly	Missense	
375		316	c.947T>C	p.Phe316Ser	Missense	
376		318	c.953C>T	p.Ser318Phe	Missense	
377		320	c.958C>T	p.Arg320*	Nonsense	10-15%
378		320	c.959G>T	p.Arg320Leu	Missense	[3.9%]
379		321	c.962C>A	p.Ser321*	Nonsense	
380		322	c.964C>G	p.Leu322Val	Missense	
381		322	c.965T>C	p.Leu322Pro	Missense	
382		323	c.967G>A	p.Val323Met	Missense	
383		326	c.976G>A	p.Glu326Lys	Missense	
384		328	c.982G>T	p.Glu328*	Nonsense	
385		330	c.988A>T; 989_990delG A	p.fsX	Frameshift	
386		330	c.988A>G	p.Arg330Gly	Missense	
387		331	c.991A>T	p.Lys331*	Nonsense	
388		332	c.994T>A	p.Trp332Arg	Missense	0%
389		332	c.995G>A	p.Trp332*	Nonsense	
390		332	c.996G>A	p.Trp332*	Nonsense	
391		332	c.995G>C	p.Trp332Ser	Missense	
392		335	c.1005G>A	p.Met335Ile	Missense	
393	Intron 9		c.1005+1G>T		Splice site error	
394			c.1005+2T>C		Splice site error	
395			c.1006-3C>G		Splice site error	2.70%
396			c.1006-1G>A		Splice site error	[23%]
397			c.1005_1091 C>G		Splice site error	
398	Exon 10	336	c.1006G>T	p.Ala336Ser	Missense	
399		337	c.1009G>C	p.Val337Leu	Missense	<5%
400		339	c.1015G>C	p.Val339Leu	Missense	

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No.	Exon	Codon ¹	Nucleotide change ²	Amino acid or other change	Function	% Enzyme activity / [%] N ammonia incorporation ³
401		339	c.1016T>G	p.Val339Gly	Missense	
402		340	c.1018T>C	p.Ser340Pro	Missense	
403		341	c.1022T>C	p.Leu341Pro	Missense	
404		343	c.1028C>G	p.Thr343Arg	Missense	
405		343	c.1028C>A	p.Thr343Lys	Missense	
406		345	c.1033T>C	p.Tyr345His	Missense	
407		345	c.1033T>G	p.Tyr345Asp	Missense	
408		345	c.1034A>G	p.Tyr345Cys	Missense	
409		347	c.1039C>A	p.Pro347Thr	Missense	
410		347	c.1039C>T	p.Pro347Ser	Missense	
411		347	c.1040C>T	p.Pro347Leu	Missense	
412		348	c.1042C>T	p.Gln348*	Nonsense	
413		348	c.1043delA	p.Gln348Argfs*47	Frameshift	
414		348	c.1046T>C	p.Leu349Pro	Missense	
415		354	c.1061T>G	p.Phe354Cys	Missense	1.80%
416		355	c.1063T>C	p.*355Glu	Extending	
417		355	c.1065A>T	p.*355Cysext*14	Extending	

Table 21. CAS Registry Numbers for Selected Compounds

Drugs	CAS Registry No
Baricitinib	1187594-09-7
Momelotinib	1056634-68-4
17-AAG	75747-14-7
BIIB021	848695-25-0
NVP-HSP990/HSP990	934343-74-5
Retaspimycin Hydrochloride	857402-63-2
Retaspimycin	857402-23-4
BIRB796/Doramapimod	285983-48-4
Pamapimod/Ro4402257/ R1503	449811-01-2
PH-797804	586379-66-0
Mubritinib/TAK-165	366017-09-6
XL-228	898280-07-4
Lifirafenib/BGB-283	1446090-79-4
BMS-214662	195987-41-8
Foretinib	849217-64-7
BX795	702675-74-9
Dorsomorphin dihydrochloride	1219168-18-9
Dorsomorphin	866405-64-3

CLAIMS

1. A method for increasing OTC gene expression in a cell harboring an OTC mutation associated with a partial reduction of OTC function, comprising: contacting the cell with an effective amount of a compound that inhibits a target selected from the group consisting of JAK1, JAK2, JAK3, HSP90, MAPK, EGFR, FGFR, BRAF, RAF1, KDR, FLT1, TBK1, IKBKE, PRKAA1, PRKAA2, PRKAB1, BMPR1A and BMPR1B.
2. The method of claim 1, wherein the cell is a hepatocyte.
3. The method of any one of claims 1-2, wherein the target is JAK1, JAK2 and JAK3 and the compound selected from the group consisting of Momelotinib and Baricitinib.
4. The method of claim 3, wherein the compound is Momelotinib.
5. The method of claim 3, wherein the compound is Baricitinib.
6. The method of any one of claims 1-2, wherein the target is HSP90 and the compound is selected from the group consisting of 17-AAG, BIIB021, HSP-990, and Retaspimycin HCl.
7. The method of any one of claims 1-2, wherein the target is MAPK and the compound is selected from the group consisting of BIRB796, Pamapimod and PH-797804.
8. The method of any one of claims 1-2, wherein the target is EGFR and the compound is Mubritinib (TAK 165).
9. The method of any one of claims 1-2, wherein the target is FGFR and the compound is XL228.
10. The method of any one of claims 1-2, wherein the target is BRAF or RAF1 and the compound is selected from the group consisting of Lifirafenib (BGB-283) and BMS-214662.
11. The method of any one of claims 1-2, wherein the target is KDR or FLT1 and the compound is Foretinib/XL880 (GSK1363089).
12. The method of any one of claims 1-2, wherein the target is TBK1 or IKBKE and the compound is BX795.
13. The method of any one of claims 1-2, wherein the target is PRKAA1, PRKAA2, or PRKAB1 and the compound is Dorsomorphin.
14. A method for increasing OTC gene expression in a cell harboring an OTC mutation associated with a partial reduction of OTC function, comprising: contacting the cell with an siRNA compound that inhibits a target selected from the group consisting of JAK1, WSTR1, YAP1, CSF1R, LYN, SMAD3, NTRK1, EPHB3, EPHB4, FGFR4, INSR, KDR, FLT1, FGFR2,

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EPHB2, PDGFRB, IRF5, FGFR1, EPHB1, FYN, FLT4, YY1, IRF1, IGF-1, SMAD1, DDR1, HSP90AA1, and SMAD2.

15. A method for increasing OTC expression in a human subject harboring an OTC mutation associated with a partial reduction of OTC function, comprising: administering to the subject an effective amount of a compound that inhibits a target selected from the group consisting of JAK1, JAK2, JAK3, HSP90, MAPK, EGFR, FGFR, BRAF, RAF1, KDR, FLT1, TBK1, IKBKE, PRKAA1, PRKAA2, PRKAB1, BMPR1A and BMPR1B.
16. The method of claim 15, wherein the target is JAK1, JAK2 or JAK3 and the compound selected from the group consisting of Momelotinib and Baricitinib.
17. The method of claim 16, wherein the compound is Momelotinib.
18. The method of claim 16, wherein the compound is Baricitinib.
19. The method of claim 15, wherein the target is HSP90 and the compound is selected from the group consisting of 17-AAG, BIIB021, HSP-990, and Retaspimycin HCl.
20. The method of claim 15, wherein the target is MAPK and the compound is selected from the group consisting of BIRB796, Pamapimod and PH-797804.
21. The method of claim 15, wherein the target is EGFR and the compound is Mubritinib (TAK 165).
22. The method of claim 15, wherein the target is FGFR and the compound is XL228.
23. The method of claim 15, wherein the target is BRAF or RAF1 and the compound is selected from the group consisting of Lifirafenib (BGB-283) and BMS-214662.
24. The method of claim 15, wherein the target is KDR or FLT1 and the compound is Foretinib/XL880 (GSK1363089).
25. The method claim 15, wherein the target is TBK1 or IKBKE and the compound is BX795.
26. The method of claim 15, wherein the target is PRKAA1, PRKAA2, or PRKAB1 and the compound is Dorsomorphin.
27. The method of any one of claims 1-26 wherein the OTC mutation is selected from the group consisting of the mutations appearing in Table 20 that are associated with non-zero percent enzyme activity.

FIG. 1

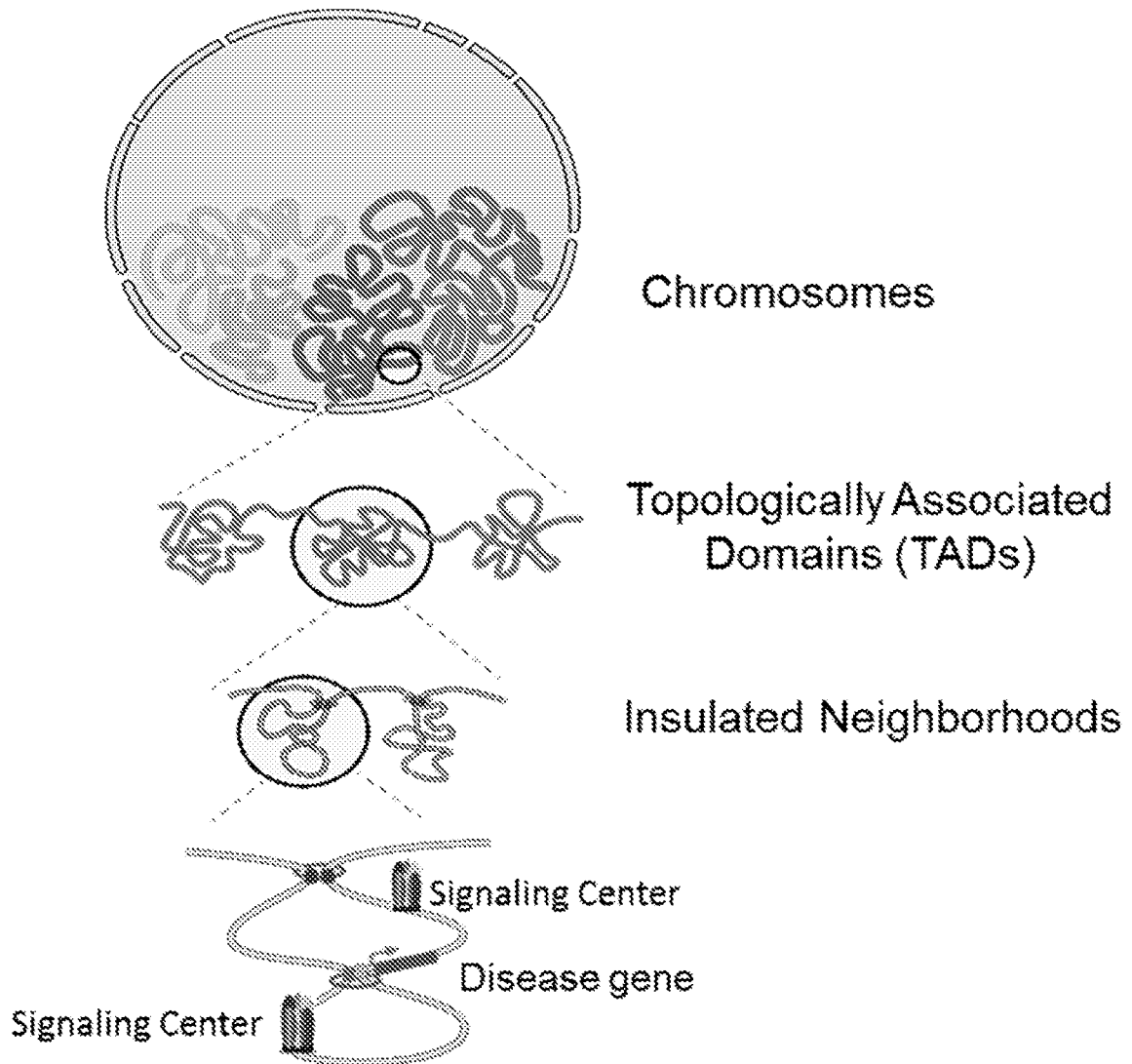


FIG. 2A



FIG. 2B

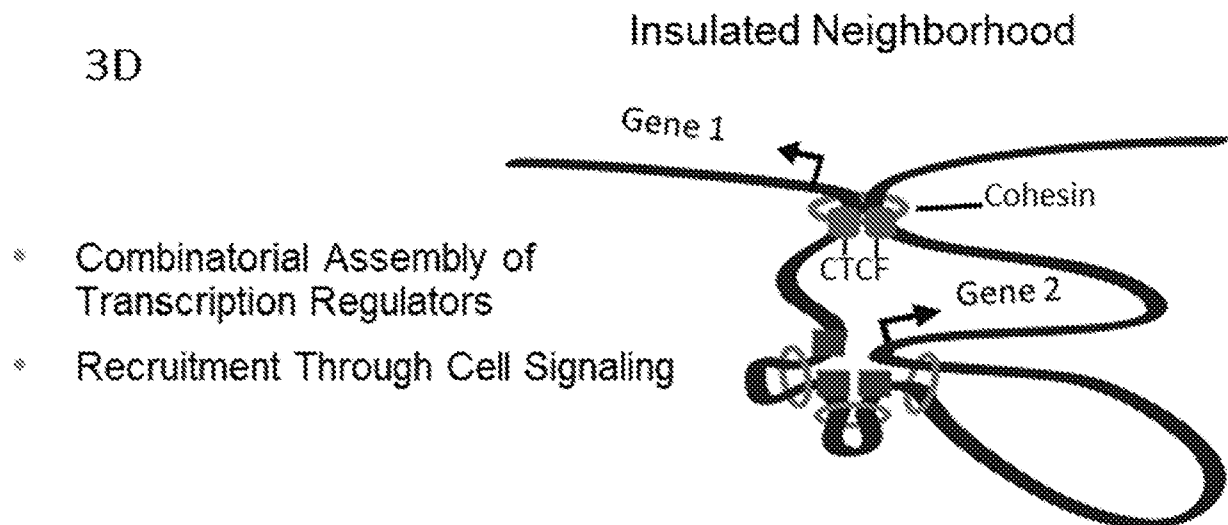


FIG. 3A

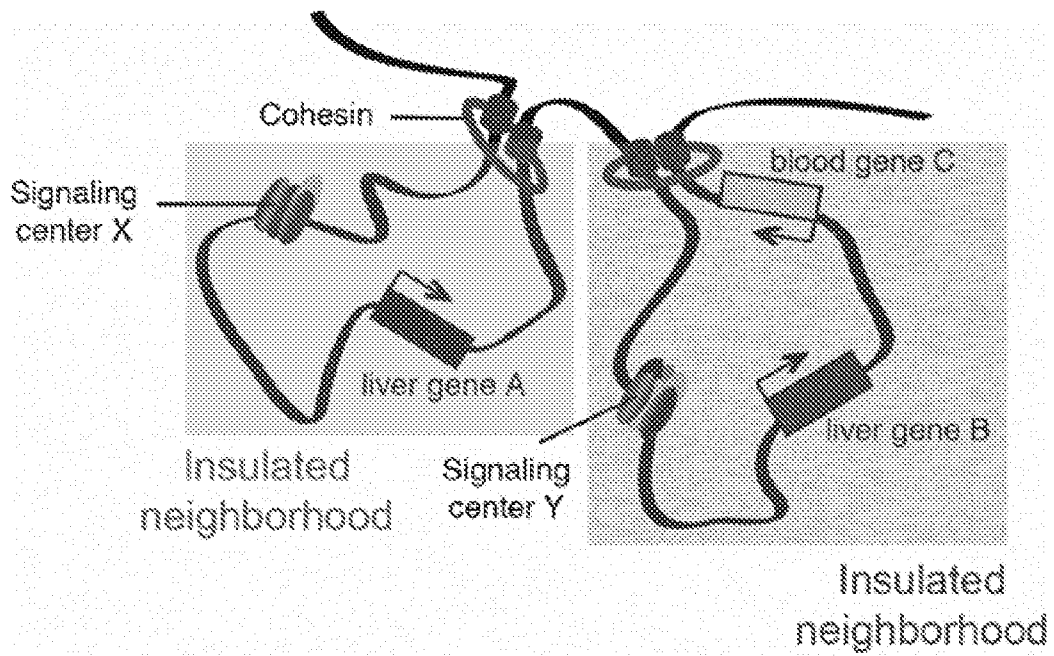


FIG. 3B

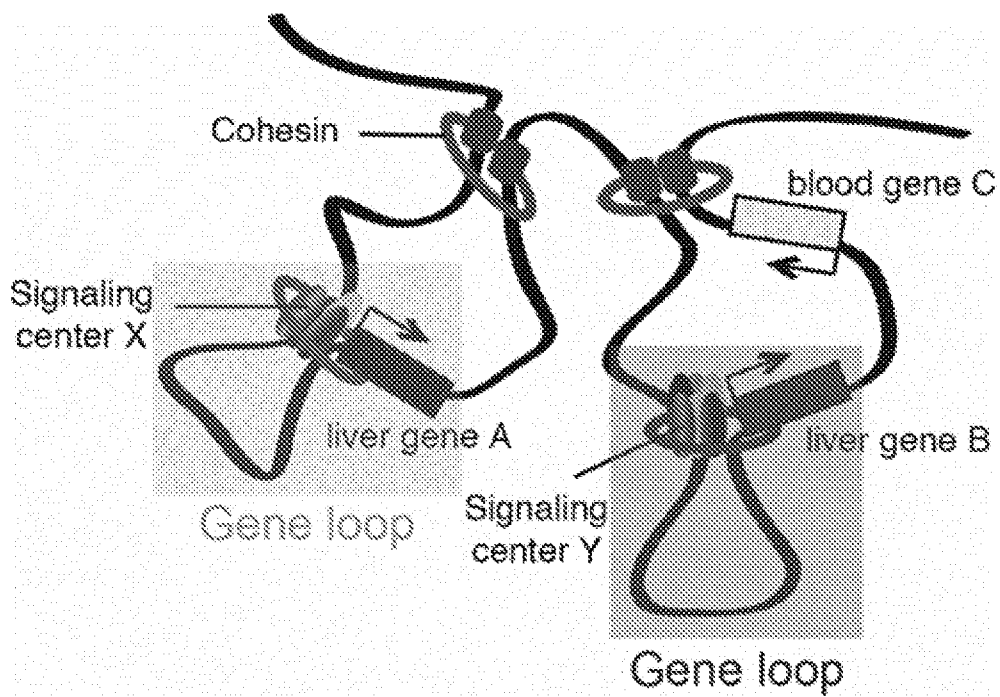


FIG. 4

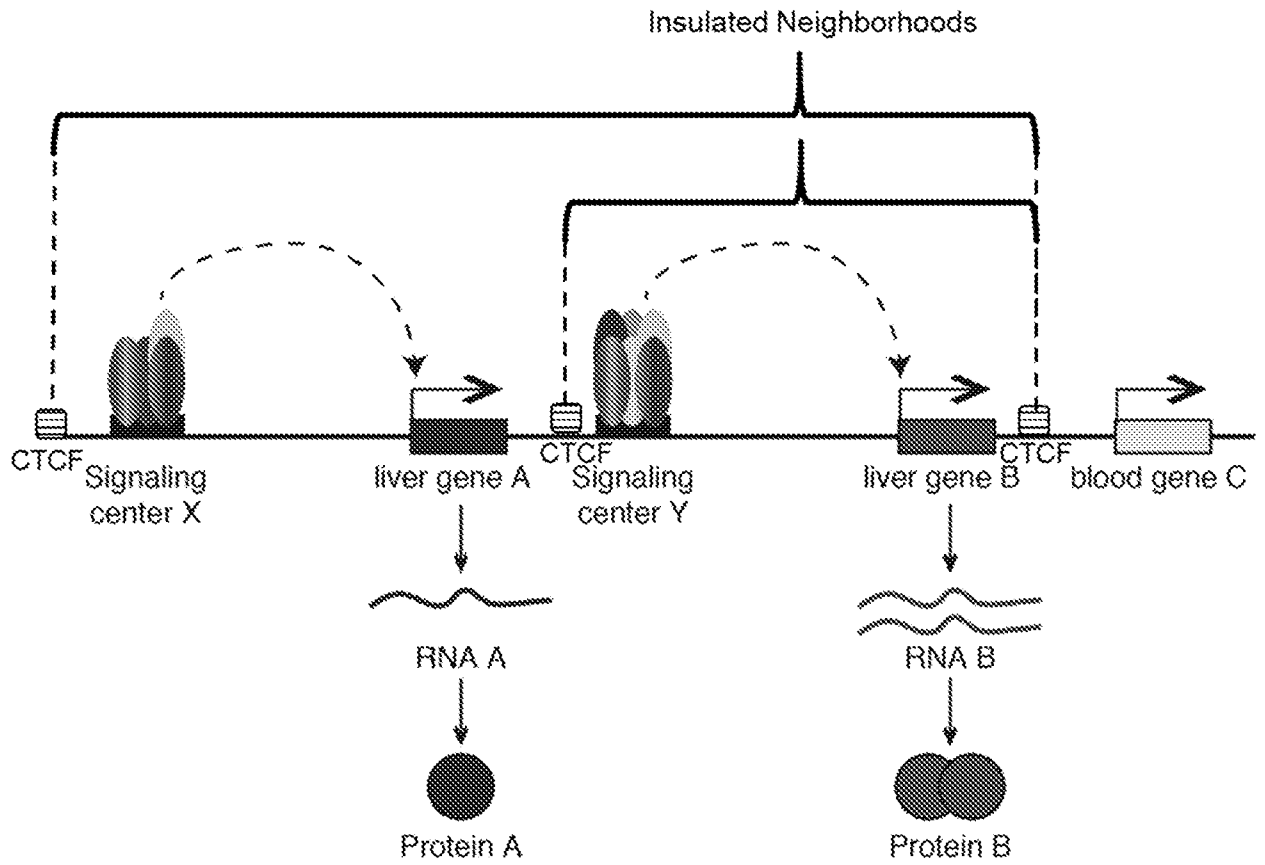


FIG. 5

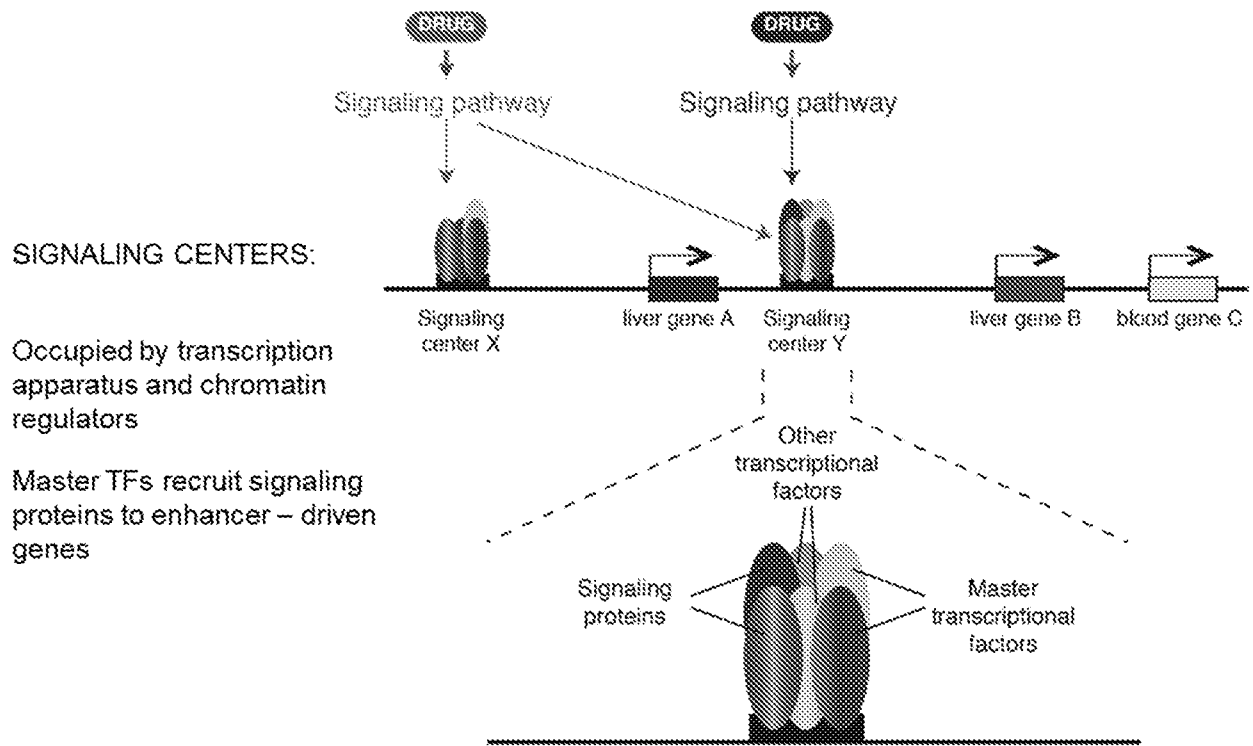


FIG. 1

