

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 August 2008 (21.08.2008)

PCT

(10) International Publication Number
WO 2008/100376 A2

(51) International Patent Classification:
C07K 14/43 (2006.01)

(74) Agents: VINCENT, Matthew, P. et al.; Ropes & Gray LLP, One International Place, Boston, MA 02110 (US).

(21) International Application Number:
PCT/US2008/001032

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(22) International Filing Date: 25 January 2008 (25.01.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/901,594 15 February 2007 (15.02.2007) US

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (for all designated States except US): SIRTRIS PHARMACEUTICALS, INC. [US/US]; 200 Technology Square, Suite 300, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): JIN, Lei [US/US]; 8 Harris Ave., Wellesley, MA 02481 (US). KORKHIN, Yakov [IL/US]; 20 Egmont, Apt. #5, Brookline, MA 02446 (US).

Published:

— without international search report and to be republished upon receipt of that report

(54) Title: TRUNCATION VARIANTS OF SIRT1 AND METHODS OF USE THEREOF

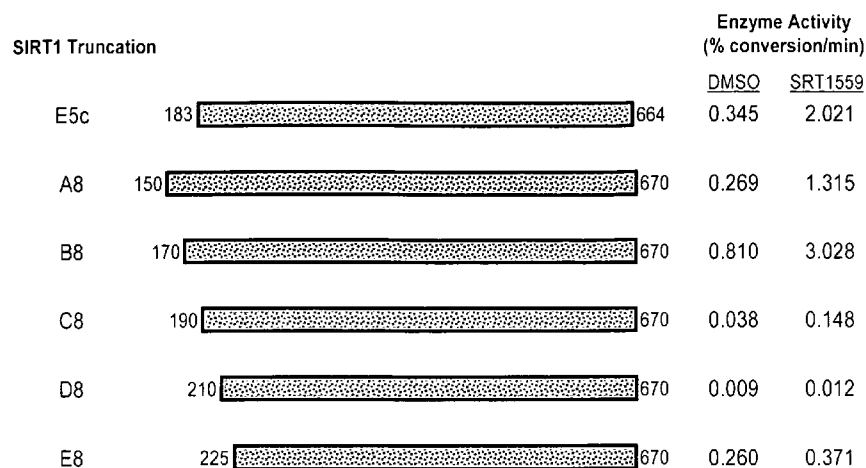


FIGURE 3

(57) Abstract: Provided herein are novel sirtuin variants and methods of use thereof. The sirtuin variants may be used for increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity. Also provided are compositions comprising sirtuin variants, optionally in combination with other therapeutic agents.

WO 2008/100376 A2

TRUNCATION VARIANTS OF SIRT1 AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/901,594,
5 filed February 15, 2007, which application is hereby incorporated by reference in its entirety.

BACKGROUND

The Silent Information Regulator (SIR) family of genes represents a highly
10 conserved group of genes present in the genomes of organisms ranging from archaeobacteria to a variety of eukaryotes (Frye, 2000). The encoded SIR proteins are involved in diverse processes from regulation of gene silencing to DNA repair. The proteins encoded by members of the SIR gene family show high sequence conservation in a 250 amino acid core domain. A well-characterized gene in this
15 family is *S. cerevisiae* SIR2, which is involved in silencing HM loci that contain information specifying yeast mating type, telomere position effects and cell aging (Guarente, 1999; Kaeberlein et al., 1999; Shore, 2000). The yeast Sir2 protein belongs to a family of histone deacetylases (reviewed in Guarente, 2000; Shore, 2000). The Sir2 homolog, CobB, in *Salmonella typhimurium*, functions as an NAD
20 (nicotinamide adenine dinucleotide)-dependent ADP-ribosyl transferase (Tsang and Escalante-Semerena, 1998).

The Sir2 protein is a class III deacetylase which uses NAD as a cosubstrate (Imai et al., 2000; Moazed, 2001; Smith et al., 2000; Tanner et al., 2000; Tanny and Moazed, 2001). Unlike other deacetylases, many of which are involved in gene
25 silencing, Sir2 is insensitive to class I and II histone deacetylase inhibitors like trichostatin A (TSA) (Imai et al., 2000; Landry et al., 2000a; Smith et al., 2000).

Deacetylation of acetyl-lysine by Sir2 is tightly coupled to NAD hydrolysis, producing nicotinamide and a novel acetyl-ADP ribose compound (Tanner et al., 2000; Landry et al., 2000b; Tanny and Moazed, 2001). The NAD-dependent
30 deacetylase activity of Sir2 is essential for its functions which can connect its biological role with cellular metabolism in yeast (Guarente, 2000; Imai et al., 2000; Lin et al., 2000; Smith et al., 2000). Mammalian Sir2 homologs have NAD-

dependent histone deacetylase activity (Imai et al., 2000; Smith et al., 2000). Most information about Sir2 mediated functions comes from the studies in yeast (Gartenberg, 2000; Gottschling, 2000).

Biochemical studies have shown that Sir2 can readily deacetylate the amino-
5 terminal tails of histones H3 and H4, resulting in the formation of 1-O-acetyl-ADP-ribose and nicotinamide. Strains with additional copies of SIR2 display increased rDNA silencing and a 30% longer life span. It has recently been shown that additional copies of the *C. elegans* SIR2 homolog, sir-2.1, and the *D. melanogaster* dSir2 gene greatly extend life span in those organisms. This implies that the SIR2-
10 dependent regulatory pathway for aging arose early in evolution and has been well conserved. Today, Sir2 genes are believed to have evolved to enhance an organism's health and stress resistance to increase its chance of surviving adversity.

Caloric restriction has been known for over 70 years to improve the health and extend the lifespan of mammals (Masoro, 2000). Yeast life span, like that of
15 metazoans, is also extended by interventions that resemble caloric restriction, such as low glucose. The discovery that both yeast and flies lacking the SIR2 gene do not live longer when calorically restricted provides evidence that SIR2 genes mediate the beneficial health effects of this diet (Anderson et al., 2003; Helfand and Rogina, 2004). Moreover, mutations that reduce the activity of the yeast glucose-responsive
20 cAMP (adenosine 3'5'-monophosphate)-dependent (PKA) pathway extend life span in wild type cells but not in mutant sir2 strains, demonstrating that SIR2 is likely to be a key downstream component of the caloric restriction pathway (Lin et al., 2001).

Recently, a number of small molecule activators and inhibitors of the SIR proteins have been reported (see e.g., U.S. Patent Application Publication Nos.
25 2005/0136537 and 2005/0096256 and PCT Publication Nos. WO 2005/002555 and WO 2005/002672) and a number of uses for these compounds have been identified. For example, small molecule activators of SIR proteins were shown to extend life span in yeast and cultured human cells as well as activate SIR protein activity in
30 human cells (supra). Additionally, the small molecule SIR activators were shown to mimic calorie restriction and extend lifespan in *Caenorhabditis elegans* and *Drosophila melanogaster* (supra). Activators of the SIR proteins may therefore be useful for mimicking the effects of calorie restriction in eukaryotic cells and treating

aging-related diseases such as stroke, cardiovascular disease, arthritis, high blood pressure, or Alzheimer's disease (supra). Additionally, it has been shown that resveratrol, butein, fisetin, piceatannol, and quercetin, small molecule activators of SIR proteins, promote fat mobilization in *C. elegans*, prevent fat accumulation in *C.*
5 *elegans*, stimulate fat mobilization in mammalian cells, and inhibit adipogenesis in mammalian cells (see e.g., U.S. Patent Publication No. 2005/0171027 and PCT Publication No. WO 2005/065667). Similarly, nicotinamide, an inhibitor of SIR proteins, was shown to promote fat accumulation (supra). Additionally, resveratrol was shown to at least partially restore insulin sensitivity in insulin resistant cells
10 (supra). Activators of SIR proteins may therefore also be useful for treating or preventing insulin resistance disorders and have been suggested for uses relating to reducing weight or preventing weight gain (supra).

The human ortholog of yeast Sir2 (silent mating type information regulation 2), SIRT1, is an NAD⁺-dependent deacetylase (Imai S et al. Cold Spring Harb Symp
15 Quant Biol. 2000; 65: 297-302). The SIRT1 protein is localized in the nucleus (Luo J et al. Cell. 2001; 107(2): 137-48; Vaziri H et al. Cell. 2001; 107(2): 149-59) and interacts with and deacetylates a large number of proteins.

Unfortunately, SIRT1 is difficult to produce and isolate in quantities useful for conducting screening assays and other applications. Accordingly, a need exists
20 for novel sirtuin variants that demonstrate increased expression while retaining acetylation activity as well as the ability to be activated by sirtuin modulating compounds.

SUMMARY

25 The present invention provides new and advantageous methods, compositions, cell constructs, polynucleotides and polypeptides, and animal models related to novel sirtuin variants.

In a first aspect, the invention provides novel SIRT1 variants, wherein the variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has
30 deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound.

In a second aspect, the invention provides novel SIRT1 polynucleotides that encode SIRT1 variants, wherein the variants i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, ii) have deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound.

In still an additional aspect, the present invention provides a transgenic mammal, a majority of whose cells harbor a transgene including a nucleic acid sequence encoding a variant of SIRT1. In an embodiment of the transgenic mammal, the SIRT1 activity level is higher in the cells of the transgenic mammal than in the cells of a nontransgenic mammal of the same species. In advantageous embodiments, the life span of those cells in the transgenic mammal that express a SIRT1 variant is increased with respect to a non-transgenic mammal of the same species. In an additional embodiment, the heterologous nucleic acid further includes one or more of an enhancer sequence, a promoter sequence, and a polyadenylation sequence each of which is operably linked to the SIRT1 sequence.

One aspect of the invention provides for a method for identifying a compound that modulates SIRT1 activity, comprising: (a) contacting a peptide substrate pool with a SIRT1 variant in the presence of a test compound, wherein members of said peptide substrate pool comprise at least one acetylated amino acid side chain and wherein the SIRT1 variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound, and (b) determining the level of acetylation of the peptide substrate pool, wherein a change in the level of acetylation of the peptide substrate pool in the presence of the test compound as compared to a control is indicative of a compound that modulates SIRT1.

Another aspect relates to a method for determining SIRT1 activity, comprising: contacting a peptide substrate pool with a SIRT1 variant, wherein members of said peptide substrate pool comprise at least one acetylated amino acid side chain, and wherein the SIRT1 variant (i) may be expressed in *E. coli* at a

concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound, and determining if the acetylated amino acid side chain in the peptide
5 substrate pool is deacetylated.

A further aspect of the invention provides for a method of deacetylating at least one amino acid residue in a polypeptide comprising the step of preparing a mixture by combining a polypeptide having at least one acetylated amino acid with a SIRT1 variant, wherein the SIRT1 variant (i) may be expressed in *E. coli* at a
10 concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound.

In another aspect, the invention provides methods for using SIRT1 variants,
15 or compositions comprising SIRT1 variants. In certain embodiments, SIRT1 variants that increase the level and/or activity of a sirtuin protein may be used for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity,
20 neurodegenerative diseases, chemotherapeutic induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. SIRT1 variants may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for enhancing muscle performance, for
25 increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia. As described further below, the methods comprise administering to a subject in need thereof a pharmaceutically effective amount of SIRT1 variants.

In certain aspects, the SIRT1 variants may be administered alone or in
30 combination with other compounds, including other SIRT1 variants, or other therapeutic agents.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows a mass spectrometry isobologram.

Figure 2 shows a series of SIRT1 N- and C-terminal truncations.

Figure 3 shows a depiction of SIRT1 N-terminal truncations that define the
5 allosteric compound binding site.

Figure 4 shows the nucleotide (SEQ ID NO: 2) and amino acid (SEQ ID NO: 1) sequences for human SIRT1. This amino acid sequence corresponds to Genbank Accession No. NP_036370 and the nucleotide sequence corresponds to a portion of Genbank Accession No. NM_012238.

10 Figure 5 is an alignment of the amino acid sequences for human SIRT1 (SEQ ID NO: 1), SIRT2 (SEQ ID NO: 7), SIRT3 (SEQ ID NO: 8), SIRT4 (SEQ ID NO: 9), SIRT5 (SEQ ID NO: 10), SIRT6 (SEQ ID NO: 11), SIRT7 (SEQ ID NO: 12), yeast Sir2 (SEQ ID NO: 5), and mouse SIRT1 (SEQ ID NO: 6).

15 DETAILED DESCRIPTION

1. Definitions

As used herein, the following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art.

20 The singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

The term "activation" refers the ability of a compound to increase the deacetylation activity of SIRT1.

The term "agent" is used herein to denote a chemical compound, a mixture
25 of chemical compounds, a biological macromolecule (such as a nucleic acid, an antibody, a protein or portion thereof, e.g., a peptide), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. The activity of such agents may render it suitable as a "therapeutic agent" which is a biologically, physiologically, or pharmacologically
30 active substance (or substances) that acts locally or systemically in a subject.

The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included.

The term "conserved residue" refers to an amino acid that is a member of a group of amino acids having certain common properties. The term "conservative amino acid substitution" refers to the substitution (conceptually or otherwise) of an amino acid from one such group with a different amino acid from the same group.

5 A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and R. H. Schirmer., Principles of Protein Structure, Springer-Verlag). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with

10 each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and R. H. Schirmer, Principles of Protein Structure, Springer-Verlag). One example of a set of amino acid groups defined in this manner include: (i) a charged group, consisting of Glu and Asp, Lys, Arg and His, (ii) a positively-charged group, consisting of Lys, Arg and His, (iii) a negatively-charged

15 group, consisting of Glu and Asp, (iv) an aromatic group, consisting of Phe, Tyr and Trp, (v) a nitrogen ring group, consisting of His and Trp, (vi) a large aliphatic nonpolar group, consisting of Val, Leu and Ile, (vii) a slightly-polar group, consisting of Met and Cys, (viii) a small-residue group, consisting of Ser, Thr, Asp, Asn, Gly, Ala, Glu, Gln and Pro, (ix) an aliphatic group consisting of Val, Leu, Ile,

20 Met and Cys, and (x) a small hydroxyl group consisting of Ser and Thr.

The term "deacetylation activity" refers to the NAD^+ dependent deacetylation enzymatic activity of SIRT1.

"Diabetes" refers to high blood sugar or ketoacidosis, as well as chronic, general metabolic abnormalities arising from a prolonged high blood sugar status or

25 a decrease in glucose tolerance. "Diabetes" encompasses both the type I and type II (Non Insulin Dependent Diabetes Mellitus or NIDDM) forms of the disease. The risk factors for diabetes include the following factors: waistline of more than 40 inches for men or 35 inches for women, blood pressure of 130/85 mmHg or higher, triglycerides above 150 mg/dl, fasting blood glucose greater than 100 mg/dl or high-

30 density lipoprotein of less than 40 mg/dl in men or 50 mg/dl in women.

A "direct activator" of a sirtuin is a molecule that activates a sirtuin by binding to it. A "direct inhibitor" of a sirtuin is a molecule inhibits a sirtuin by binding to it.

5 A "fusion protein" or "fusion polypeptide" refers to a chimeric protein as that term is known in the art and may be constructed using methods known in the art. In many examples of fusion proteins, there are two different polypeptide sequences, and in certain cases, there may be more. The sequences may be linked in frame. A fusion protein may include a domain which is found (albeit in a different protein) in an organism which also expresses the first protein, or it may be an "interspecies",
10 "intergenic", etc. fusion expressed by different kinds of organisms. In various embodiments, the fusion polypeptide may comprise one or more amino acid sequences linked to a first polypeptide. In the case where more than one amino acid sequence is fused to a first polypeptide, the fusion sequences may be multiple copies of the same sequence, or alternatively, may be different amino acid sequences. The
15 fusion polypeptides may be fused to the N-terminus, the C-terminus, or the N- and C-terminus of the first polypeptide. Exemplary fusion proteins include polypeptides comprising a glutathione S-transferase tag (GST-tag), histidine tag (His-tag), an immunoglobulin domain, an immunoglobulin binding domain, or an amino acid sequence which promotes transcytosis.

20 The term "hyperinsulinemia" refers to a state in an individual in which the level of insulin in the blood is higher than normal.

The term "including" is used to mean "including but not limited to". "Including" and "including but not limited to" are used interchangeably.

25 The term "insulin resistance" refers to a state in which a normal amount of insulin produces a subnormal biologic response relative to the biological response in a subject that does not have insulin resistance.

30 An "insulin resistance disorder," as discussed herein, refers to any disease or condition that is caused by or contributed to by insulin resistance. Examples include: diabetes, obesity, metabolic syndrome, insulin-resistance syndromes, syndrome X, insulin resistance, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, hyperlipidemia, dyslipidemia, atherosclerotic disease

including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release, diabetic complications, including coronary heart disease, angina pectoris, congestive heart failure, stroke, cognitive functions in dementia, 5 retinopathy, peripheral neuropathy, nephropathy, glomerulonephritis, glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation, polycystic ovarian syndrome (PCOS)), lipodystrophy, 10 cholesterol related disorders, such as gallstones, cholecystitis and cholelithiasis, gout, obstructive sleep apnea and respiratory problems, osteoarthritis, and prevention and treatment of bone loss, e.g. osteoporosis.

The term "isolated nucleic acid" refers to a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which (1) is not associated 15 with the cell in which the "isolated nucleic acid" is found in nature, or (2) is operably linked to a polynucleotide to which it is not linked in nature.

The term "livestock animals" refers to domesticated quadrupeds, which includes those being raised for meat and various byproducts, e.g., a bovine animal including cattle and other members of the genus *Bos*, a porcine animal including 20 domestic swine and other members of the genus *Sus*, an ovine animal including sheep and other members of the genus *Ovis*, domestic goats and other members of the genus *Capra*; domesticated quadrupeds being raised for specialized tasks such as use as a beast of burden, e.g., an equine animal including domestic horses and other members of the family *Equidae*, genus *Equus*.

25 The term "mammal" is known in the art, and exemplary mammals include humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

An "NAD-like compound" is also within the scope of the invention and refers to a compound (e.g., a synthetic or naturally occurring chemical, drug, 30 protein, peptide, small organic molecule) which possesses structural similarity (e.g., adenine, ribose and phosphate groups) or functional similarity (e.g., oxidation of substrates, NAD-dependent deacetylation of histone proteins). For example, NAD-

like compounds can be NADH, NADP, NADPH, non-hydrolyzable NAD and fluorescent analogs of NAD (e.g., 1, N6-etheno NAD).

“Obese” individuals or individuals suffering from obesity are generally individuals having a body mass index (BMI) of at least 25 or greater. Obesity may
5 or may not be associated with insulin resistance.

The terms “parenteral administration” and “administered parenterally” are art-recognized and refer to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac,
10 intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articulare, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

A “patient”, “subject”, “individual” or “host” refers to either a human or a non-human animal.

15 The term “percent identical” refers to sequence identity between two amino acid sequences or between two nucleotide sequences. Identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base or amino acid, then the molecules are identical at that
20 position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Expression as a
25 percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Various alignment algorithms and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be
30 used with, e.g., default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD. In one embodiment, the percent identity of two sequences

can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid or nucleotide mismatch between the two sequences.

Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Preferably, an alignment program that permits gaps in the sequence is utilized to align the sequences. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. An alternative search strategy uses MPSRCH software, which runs on a MASPAR computer. MPSRCH uses a Smith-Waterman algorithm to score sequences on a massively parallel computer. This approach improves ability to pick up distantly related matches, and is especially tolerant of small gaps and nucleotide sequence errors. Nucleic acid-encoded amino acid sequences can be used to search both protein and DNA databases.

The term "pharmaceutically acceptable carrier" is art-recognized and refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof. Each carrier must be "acceptable" in the sense of being compatible with the subject composition and its components and not injurious to the patient. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18)

Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The terms "polynucleotide", and "nucleic acid" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either
5 deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, exons, introns, messenger RNA (mRNA), cDNA, recombinant polynucleotides, branched polynucleotides, plasmids,
10 vectors, isolated DNA of any sequence, and isolated RNA of any sequence. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further
15 modified, such as by conjugation with a labeling component. The term "recombinant" polynucleotide means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a nonnatural arrangement.

The term "prophylactic" or "therapeutic" treatment is art-recognized and
20 refers to administration of a drug to a host. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish,
25 ameliorate or maintain the existing unwanted condition or side effects therefrom).

The term "pyrogen-free", with reference to a composition, refers to a composition that does not contain a pyrogen in an amount that would lead to an adverse effect (e.g., irritation, fever, inflammation, diarrhea, respiratory distress, endotoxic shock, etc.) in a subject to which the composition has been administered.
30 For example, the term is meant to encompass compositions that are free of, or substantially free of, an endotoxin such as, for example, a lipopolysaccharide (LPS).

The terms "recombinant protein" or "recombinant polypeptide" refer to a polypeptide which is produced by recombinant DNA techniques. An example of such techniques includes the case when DNA encoding the expressed protein is inserted into a suitable expression vector which is in turn used to transform a host
5 cell to produce the protein or polypeptide encoded by the DNA.

"Replicative lifespan" of a cell refers to the number of daughter cells produced by an individual "mother cell." "Chronological aging" or "chronological lifespan," on the other hand, refers to the length of time a population of non-dividing cells remains viable when deprived of nutrients. "Increasing the lifespan
10 of a cell" or "extending the lifespan of a cell," as applied to cells or organisms, refers to increasing the number of daughter cells produced by one cell; increasing the ability of cells or organisms to cope with stresses and combat damage, e.g., to DNA, proteins; and/or increasing the ability of cells or organisms to survive and exist in a living state for longer under a particular condition, e.g., stress (for
15 example, heatshock, osmotic stress, high energy radiation, chemically-induced stress, DNA damage, inadequate salt level, inadequate nitrogen level, or inadequate nutrient level). Lifespan can be increased by at least about 20%, 30%, 40%, 50%, 60% or between 20% and 70%, 30% and 60%, 40% and 60% or more using methods described herein. Techniques to assess the life span cells are well known
20 and are described, for example, in Guarente et al., U.S. Patent No. 5,874,210 (1999); Hayflick et al., Experimental Cell Research, 25:585-621, (1961); Todaro, et al., Journal of Cell Biology, 17: 299-313, (1963); Rohme, Proc. Natl. Acad. Sci. USA, 78:5009-5013, (1981).

"Sirtuin-activating compound," as used herein, refers to a compound that
25 increases the level of deacetylase activity of a sirtuin protein. In an exemplary embodiment, a sirtuin-activating compound may increase deacetylase activity of a sirtuin protein by at least about 10%, 25%, 50%, 75%, 100%, or more. Sirtuin deacetylation activity may be determined using a variety of substrates for sirtuin proteins. Examples of sirtuin substrates include, for example, histones and p53, or
30 fragments thereof. Exemplary sirtuin activating compounds include flavones, stilbenes, flavanones, isoflavanones, catechins, chalcones, tannins and

anthocyanidins. Exemplary stilbenes include hydroxystilbenes, such as trihydroxystilbenes, e.g., 3,5,4'-trihydroxystilbene ("resveratrol"). Resveratrol is also known as 3,4',5-stilbenetriol. Tetrahydroxystilbenes, e.g., piceatannol, are also encompassed. Hydroxychalcones including trihydroxychalcones, such as isoliquiritigenin, and tetrahydroxychalcones, such as butein, can also be used.

5 Hydroxyflavones including tetrahydroxyflavones, such as fisetin, and pentahydroxyflavones, such as quercetin, can also be used. Other sirtuin activating compounds are described herein below and in U.S. Patent Application Publication No. 2005/0096256 and PCT Application Nos. PCT/US06/002092,

10 PCT/US06/007746, PCT/US06/007744, PCT/US06/007745, PCT/US06/007778, PCT/US06/007656, PCT/US06/007655 and PCT/US06/007773.

"Sirtuin protein" refers to a member of the sirtuin deacetylase protein family, or preferably to the sir2 family, which include yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912),

15 and human SIRT1 (GenBank Accession No. NM_012238 and NP_036370 (or AF083106)) and SIRT2 (GenBank Accession No. NM_012237, NM_030593, NP_036369, NP_085096, and AF083107) proteins. Other family members include the four additional yeast Sir2-like genes termed "*HST* genes" (homologues of *Sir* two) HST1, HST2, HST3 and HST4, and the six other human homologues hSIRT2,

20 hSIRT3, hSIRT4, hSIRT5, hSIRT6 and hSIRT7 (Brachmann et al. (1995) *Genes Dev.* 9:2888 and Frye et al. (1999) *BBRC* 260:273). Preferred sirtuins are those that share more similarities with SIRT1, i.e., hSIRT1, and/or Sir2 than with SIRT2, such as those members having at least part of the N-terminal sequence present in SIRT1 and absent in SIRT2 such as SIRT3 has.

25 "SIRT1 protein" refers to a member of the sir2 family of sirtuin deacetylases. In one embodiment, a SIRT1 protein includes yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912), human SIRT1 (SEQ ID NO: 1), and human SIRT2 (GenBank Accession No. NM_012237, NM_030593, NP_036369, NP_085096, or AF083107) proteins, and equivalents

30 thereof. In another embodiment, a SIRT1 protein includes a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set

forth in SEQ ID NO: 1 or GenBank Accession Nos. NP_501912, NP_085096, NP_036369, or P53685. SIRT1 proteins include polypeptides comprising all or a portion of the amino acid sequence set forth in SEQ ID NO: 1 or GenBank Accession Nos. NP_501912, NP_085096, NP_036369, or P53685; the amino acid
5 sequence set forth in SEQ ID NO: 1 or GenBank Accession Nos. NP_501912, NP_085096, NP_036369, or P53685 with 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 75 or more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1 or GenBank Accession Nos. NP_501912, NP_085096, NP_036369, or P53685

10 The term "specifically hybridizes" refers to detectable and specific nucleic acid binding. Polynucleotides, oligonucleotides and nucleic acids of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. Stringent conditions may be used to achieve selective hybridization conditions
15 as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and nucleic acids of the invention and a nucleic acid sequence of interest will be at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or more. In certain instances, hybridization and washing conditions are performed under stringent conditions
20 according to conventional hybridization procedures and as described further herein.

The terms "stringent conditions" or "stringent hybridization conditions" refer to conditions which promote specific hybridization between two complementary polynucleotide strands so as to form a duplex. Stringent conditions may be selected to be about 5° C lower than the thermal melting point (T_m) for a given
25 polynucleotide duplex at a defined ionic strength and pH. The length of the complementary polynucleotide strands and their GC content will determine the T_m of the duplex, and thus the hybridization conditions necessary for obtaining a desired specificity of hybridization. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the a polynucleotide sequence hybridizes to a perfectly
30 matched complementary strand. In certain cases it may be desirable to increase the stringency of the hybridization conditions to be about equal to the T_m for a particular duplex.

A variety of techniques for estimating the T_m are available. Typically, G-C base pairs in a duplex are estimated to contribute about 3° C to the T_m , while A-T base pairs are estimated to contribute about 2° C, up to a theoretical maximum of about 80-100° C. However, more sophisticated models of T_m are available in which
5 G-C stacking interactions, solvent effects, the desired assay temperature and the like are taken into account. For example, probes can be designed to have a dissociation temperature (T_d) of approximately 60° C., using the formula:
$$T_d = (((((3 \times \#GC) + (2 \times \#AT)) \times 37) - 562) / \#bp) - 5;$$
 where #GC, #AT, and #bp are the number of guanine-cytosine base pairs, the number of adenine-thymine base pairs,
10 and the number of total base pairs, respectively, involved in the formation of the duplex.

Hybridization may be carried out in 5xSSC, 4xSSC, 3xSSC, 2xSSC, 1xSSC or 0.2xSSC for at least about 1 hour, 2 hours, 5 hours, 12 hours, or 24 hours. The temperature of the hybridization may be increased to adjust the stringency of the
15 reaction, for example, from about 25° C. (room temperature), to about 45° C, 50° C, 55° C, 60° C, or 65° C. The hybridization reaction may also include another agent affecting the stringency, for example, hybridization conducted in the presence of 50% formamide increases the stringency of hybridization at a defined temperature.

The hybridization reaction may be followed by a single wash step, or two or
20 more wash steps, which may be at the same or a different salinity and temperature. For example, the temperature of the wash may be increased to adjust the stringency from about 25° C (room temperature), to about 45° C, 50° C, 55° C, 60° C, 65° C, or higher. The wash step may be conducted in the presence of a detergent, e.g., 0.1 or 0.2% SDS. For example, hybridization may be followed by two wash steps at 65° C
25 each for about 20 minutes in 2xSSC, 0.1% SDS, and optionally two additional wash steps at 65° C each for about 20 minutes in 0.2xSSC, 0.1% SDS.

Exemplary stringent hybridization conditions include overnight hybridization at 65° C in a solution comprising, or consisting of, 50% formamide, 10x Denhardt (0.2% Ficoll, 0.2% Polyvinylpyrrolidone, 0.2% bovine serum albumin) and 200
30 µg/ml of denatured carrier DNA, e.g., sheared salmon sperm DNA, followed by two

wash steps at 65° C each for about 20 minutes in 2xSSC, 0.1% SDS, and two wash steps at 65° C each for about 20 minutes in 0.2xSSC, 0.1% SDS.

Hybridization may consist of hybridizing two nucleic acids in solution, or a nucleic acid in solution to a nucleic acid attached to a solid support, e.g., a filter.

- 5 When one nucleic acid is on a solid support, a prehybridization step may be conducted prior to hybridization. Prehybridization may be carried out for at least about 1 hour, 3 hours or 10 hours in the same solution and at the same temperature as the hybridization solution (without the complementary polynucleotide strand).

- Appropriate stringency conditions are known to those skilled in the art or
10 may be determined experimentally by the skilled artisan. See, for example, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-12.3.6; Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; S. Agrawal (ed.) Methods in Molecular Biology, volume 20; Tijssen (1993) Laboratory Techniques in biochemistry and molecular biology-
15 hybridization with nucleic acid probes, e.g., part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, N.Y.; and Tibanyenda, N. et al., Eur. J. Biochem. 139:19 (1984) and Ebel, S. et al., Biochem. 31:12083 (1992).

- The terms "systemic administration," "administered systemically,"
20 "peripheral administration" and "administered peripherally" are art-recognized and refer to the administration of a subject composition, therapeutic or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes.

- The term "therapeutic effect" is art-recognized and refers to a local or
25 systemic effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The phrase "therapeutically-effective amount" means that amount of such a substance that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. The therapeutically effective amount of such substance will vary
30 depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. For

example, certain compositions described herein may be administered in a sufficient amount to produce a desired effect at a reasonable benefit/risk ratio applicable to such treatment.

The term "transgene" refers to a nucleic acid sequence, which is partly or
5 entirely heterologous to a transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its
10 insertion results in a knockout). A transgene may include one or more regulatory sequences and any other nucleic acids, such as introns, that may be necessary for optimal expression.

The term "transgenic animal" refers to any animal, for example, a mouse, rat or other non-human mammal, a bird or an amphibian, in which one or more of the
15 cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro*
20 fertilization, but rather is directed to the introduction of a recombinant DNA molecule. In the typical transgenic animals described herein, the transgene causes cells to express a recombinant form of a protein. However, transgenic animals in which the recombinant gene is silent are also contemplated.

"Treating" a condition or disease refers to curing as well as ameliorating at
25 least one symptom of the condition or disease.

The term "vision impairment" refers to diminished vision, which is often only partially reversible or irreversible upon treatment (e.g., surgery). Particularly severe vision impairment is termed "blindness" or "vision loss", which refers to a complete loss of vision, vision worse than 20/200 that cannot be improved with
30 corrective lenses, or a visual field of less than 20 degrees diameter (10 degrees radius).

2. SIRT1 Variants

In a first aspect, the invention provides sirtuin variant polypeptides that have a higher expression level than the equivalent wild-type sirtuin, have deacetylase activity that is substantially equivalent to the deacetylase activity of the equivalent wild-type sirtuin, and which have deacetylase activity that may be activated by at least 2-fold in the presence of a sirtuin activating compound. In certain embodiments, the sirtuin variants are fragments of a full-length sirtuin protein and have increased expression with respect to the full length protein while maintaining deacetylase activity that is substantially equivalent to the corresponding full length sirtuin and which deacetylase activity is activatable by a sirtuin activating compound. In an exemplary embodiment, the invention provides SIRT1 variants that may be expressed in *E. coli* at a concentration of at least 5 mg/L, have deacetylase activity that is substantially equivalent to the deacetylase activity of the equivalent wild-type SIRT1, and have deacetylase activity that may be activated by at least 2-fold in the presence of a sirtuin activating compound. In certain embodiments, the SIRT1 protein is a human SIRT1 protein.

The sirtuin variants described herein have increased expression levels as compared to the equivalent wild-type sirtuin. For example, if the sirtuin variant is a variant of human SIRT1 then the variant will have increased expression with respect to wild-type human SIRT1. Determination of the expression level of the variant as compared to the wild-type protein may be conducted by comparing the expression levels of the variant as compared to the wild-type under the same conditions, e.g., expression from the same expression vector, in the same host cell, at the same temperature, for the length of expression, and/or under the same buffer, temperature, shaking conditions, etc. In certain embodiments, expression of the variant may be at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more greater than the expression of the wild-type sirtuin under equivalent conditions. In certain embodiments, expression of the sirtuin variant as compared to the wild-type protein is determined in a bacterial host cell, such as, for example, *E. coli*. For example, a SIRT1 variant may be expressed in *E. coli* at a concentration of at least 2 mg/L, at least 5 mg/L, at least 6 mg/L, at least 8 mg/L, at least 10 mg/L, at least 12 mg/L, or at least 15 mg/L.

In certain embodiments, expression of a SIRT1 variant in *E. coli* may be determined by expressing the SIRT1 variant under the control of a T7 promoter-based expression system when expressed in *E. coli* BL21 StarTM cells (DE3) (Invitrogen) under the following conditions: cells are grown in LB media at 37 °C until the OD600 reaches 0.8, the temperature of the culture is cooled down to 16 °C on ice and IPTG is added to 1 mM, the culture is then incubated at 16 °C for 14-16 hrs and cells are harvest by centrifugation at 29,000 g for 30 min at 4 °C.

The deacetylase activity of a sirtuin variant can be measured using any suitable deacetylation assay, including for example, any of the assays described herein. The deactylase activity of the sirtuin variant may be substantially the same as the deacetylase activity of the wild-type sirtuin under equivalent assay conditions, e.g, the deacetylase activity of the sirtuin variant is at least 95%, 96%, 97%, 98%, 99% or greater of the sirtuin activity of the corresponding wild-type sirtuin under comparable conditions. In an exemplary embodiment, the sirtuin variant is a SIRT1 variant which has substantially the same deacetylase activity as wild-type SIRT1.

Activation of sirtuin deacetylase activity can be measured using any suitable deacetylation assay, including the ones described herein, in the presence of a sirtuin activating compound. In certain embodiments, the activatability of the sirtuin variant may be substantially equivalent to the activatability of the corresponding wild-type sirtuin protein, e.g., the deacetylase activity of the sirtuin variant may be activated by at least 95%, 96%, 97%, 98%, 99%, or more, of level of activation of the wild-type sirtuin protein under equivalent conditions, e.g., in the presence of the same sirtuin activating compound, using the same substrate, using the same readout, under the same assay conditions, etc. In certain embodiments, the sirtuin variant may be activated by at 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or more in the presence of a sirtuin activating compound. In certain embodiments, the sirtuin variant is activated by at least 2-fold in the presence of resveratrol or Compound #2 or Compound #3 as described herein. In an exemplary embodiment, the sirtuin variant is a SIRT1 variant.

In certain embodiments, the invention provides isolated or purified sirtuin variants, including isolated or purified SIRT1 variants. In a further embodiment the

sirtuin variant may be a recombinant variant, such as, for example, a recombinant SIRT1 variant.

In one embodiment, the sirtuin variant is a SIRT1 variant comprises, consists essentially of, or consists of, an amino acid sequence selected from the group consisting of: (i) a fragment of SEQ ID NO: 1 having an N-terminus that falls between amino acid residues 140 to 190, 149 to 169, 154 to 184, 154 to 169, 169 to 184 of SEQ ID NO: 1 and a C-terminus that falls between amino acid residues 663 to 704, 663 to 671, or 663 to 748 of SEQ ID NO:1, (ii) a fragment of SEQ ID NO: 1 having amino acids residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1, (iii) a polypeptide which is at least 90%, 95%, 96%, 97%, 98%, 99%, or more, identical to the fragment of (i) or (ii), or (iv) the fragment of (i) or (ii) with 1 to about 20, 1 to about 10, 1 to about 5, or 1 to about 3, conserved amino acid changes.

In exemplary embodiments, the SIRT1 variants of the invention comprise amino acids residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1. In another embodiment, the SIRT1 variants of the invention comprise a fragment of SEQ ID NO:1 consisting essentially of, or consisting of, amino acid residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1.

In certain embodiments, the SIRT1 variants described herein do not include a polypeptide consisting of amino acid residues 261 to 447 of SEQ ID NO: 1, a fragment consisting of amino acid residues 242 to 493 of SEQ ID NO: 1, or a fragment consisting of amino acid residues 254 to 495 of SEQ ID NO: 1.

In another embodiment, the invention provides a SIRT1 variant that is not activatable by resveratrol or other sirtuin activating compounds that bind to the same location on SIRT1 as resveratrol. Such variants may be useful, for example, to identify sirtuin activating compounds that activate SIRT1 by a different mechanism of action. In an exemplary embodiment, the unactivatable SIRT1 variant comprises at least of fragment of SEQ ID NO:1, with the proviso that the variant excludes amino acid residues 183-225 of SEQ ID NO:1.

In certain embodiments, a sirtuin variant of the invention is a fusion protein containing a domain which increases its solubility and/or facilitates its purification, identification, detection, structural characterization, and/or cellular uptake. Exemplary domains, include, for example, glutathione S-transferase (GST), protein
5 A, protein G, calmodulin-binding peptide, thioredoxin, maltose binding protein, HA, myc, poly arginine, poly His, poly His-Asp or FLAG fusion proteins and tags. Additional exemplary domains include domains that alter protein localization *in vivo*, such as signal peptides, type III secretion system-targeting peptides, transcytosis domains, nuclear localization signals, etc. In various embodiments, a
10 variant of the invention may comprise one or more heterologous fusions. Variants may contain multiple copies of the same fusion domain or may contain fusions to two or more different domains. The fusions may occur at the N-terminus of the variant, at the C-terminus of the variant, or at both the N- and C-terminus of the variant. It is also within the scope of the invention to include linker sequences
15 between a variant of the invention and the fusion domain in order to facilitate construction of the fusion protein or to optimize protein expression or structural constraints of the fusion protein. In another embodiment, the variant may be constructed so as to contain protease cleavage sites between the fusion domain and sirtuin variant in order to remove the tag after protein expression or thereafter.
20 Examples of suitable endoproteases, include, for example, Factor Xa, thrombin, enterokinase and TEV proteases.

In another embodiment, a sirtuin variant may be modified so that its rate of traversing the cellular membrane is increased. For example, the variant may be fused to a second peptide which promotes "transcytosis," e.g., uptake of the peptide by
25 cells. The peptide may be a portion of the HIV transactivator (TAT) protein, such as the fragment corresponding to residues 37-62 or 48-60 of TAT, portions which have been observed to be rapidly taken up by a cell *in vitro* (Green and Loewenstein, (1989) Cell 55:1179-1188). Alternatively, the internalizing peptide may be derived from the *Drosophila* antennapedia protein, or homologs thereof. The 60 amino acid
30 long homeodomain of the homeo-protein antennapedia has been demonstrated to translocate through biological membranes and can facilitate the translocation of heterologous polypeptides to which it is coupled. Thus, variants may be fused to a

peptide consisting of about amino acids 42-58 of *Drosophila antennapedia* or shorter fragments for transcytosis (Derossi et al. (1996) *J Biol Chem* 271:18188-18193; Derossi et al. (1994) *J Biol Chem* 269:10444-10450; and Perez et al. (1992) *J Cell Sci* 102:717-722). The transcytosis polypeptide may also be a non-naturally-
5 occurring membrane-translocating sequence (MTS), such as the peptide sequences disclosed in U.S. Pat. No. 6,248,558.

It is also possible to modify the structure of the variants of the invention for such purposes as enhancing therapeutic or prophylactic efficacy, or stability (e.g., *ex vivo* shelf life, resistance to proteolytic degradation *in vivo*, etc.). Such modified
10 variants may be expressed in *E. coli* at a concentration of at least 5 mg/L, may have deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound. Such modified variants may be produced, for instance, by amino acid substitution, deletion, or addition, which substitutions
15 may consist in whole or part by conservative amino acid substitutions.

For instance, it is reasonable to expect that an isolated conservative amino acid substitution, such as replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, will not have a major affect on the biological activity of the resulting molecule. Whether a change in the amino
20 acid sequence of a variant affects the *E. coli* expression level, the deacetylase activity, or the activation of the deacetylase activity may be readily determined using the methods described herein. Variants in which more than one replacement has taken place may readily be tested in the same manner.

In another embodiment, methods for identifying sirtuin variants are also
25 provided herein. For example, libraries of candidate sirtuin variants may be generated and tested for expression level, deacetylase activity and activatability. Suitable methods for conducting such test are described herein. The libraries may comprise, for example, a variety of truncation mutants for one or more sirtuin proteins. The truncation mutants may have sequences removed at the N-terminus,
30 the C-terminus, or both, as compared to the corresponding full length sirtuin protein. A library may comprise, for example, 10, 50, 100, 250, 500, 750, 1000 or more different members. A candidate variant library may be produced, for example, using

recombinant DNA techniques and the set of potential sirtuin variant nucleotide sequences may be expressed as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display). In exemplary embodiments, the libraries may comprise truncation mutants of a human sirtuin protein, such as, for example, hSIRT1, hSIRT2, hSIRT3, hSIRT4, hSIRT5, hSIRT6, hSIRT7, or combinations thereof. In exemplary embodiments, the libraries comprise truncation mutants of one or more sirtuin proteins that are activatable by a sirtuin activating compound, such as resveratrol, as a full length protein, such as, for example, human SIRT1, human SIRT2, human SIRT3, human SIRT5, mouse SIRT1, rat SIRT1, or yeast Sir2. In certain embodiments, the candidate sirtuin variants may comprise the deacetylase domain of a sirtuin protein (e.g., about amino acid residues 247-661, 249-663, or 250-664 of human SIRT1) and at least a portion of the sequence flanking the deacetylase domain to the N-terminus of the sirtuin protein, for example, corresponding to at least a portion of the region comprising amino acid residues 150-220 of human SIRT1. See Figure 5 for an alignment of the human sirtuin proteins along with several SIRT1 homologs (yeast Sir2, mouse SIRT1 and rat SIRT1).

In exemplary embodiments, the sirtuin variant polypeptides of the invention may be purified, for example, to at 80%, 85%, 90%, 95%, 98%, 99%, or 99.9% purity, or greater, with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. A purified sirtuin variant polypeptide may be substantially free of other polypeptides, particularly other polypeptides of animal origin.

Sirtuin variant polypeptides can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable, including, for example, DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ). Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl

groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked
5 agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation,
10 N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding polypeptides to support media are well known in the art. Selection of a particular method is a matter
15 of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988). The sirtuin variant polypeptides described herein can also be isolated an affinity tag (e.g., polyhistidine, maltose-binding protein, an immunoglobulin domain) to facilitate purification as described further
20 herein.

3. SIRT1 Nucleic Acids

In another aspect, the invention relates to nucleic acids that encode the sirtuin variant polypeptides described herein. In one embodiment, the invention relates to
25 nucleic acids that encode sirtuin variant polypeptides that have a higher expression level than the equivalent wild-type sirtuin, have deacetylase activity that is substantially equivalent to the deacetylase activity of the equivalent wild-type sirtuin, and which have deacetylase activity that may be activated by at least 2-fold in the presence of a sirtuin activating compound. In another embodiment, the
30 invention provides nucleic acids that encode SIRT1 variants that may be expressed in *E. coli* at a concentration of at least 5 mg/L, have deacetylase activity that is substantially equivalent to the deacetylase activity of the equivalent wild-type

SIRT1, and have deacetylase activity that may be activated by at least 2-fold in the presence of a sirtuin activating compound. In certain embodiments, the nucleic acids encode SIRT1 variants that are variants of a human SIRT1 protein.

In one embodiment, the nucleic acid encodes a SIRT1 variant that comprises,
5 consists essentially of, or consists of, an amino acid sequence selected from the group consisting of: (i) a fragment of SEQ ID NO: 1 having an N-terminus that falls between amino acid residues 140 to 190, 149 to 169, 154 to 184, 154 to 169, 169 to 184 of SEQ ID NO: 1 and a C-terminus that falls between amino acid residues 663 to 704, 663 to 671, or 663 to 748 of SEQ ID NO:1, (ii) a fragment of SEQ ID NO: 1
10 having amino acids residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1, (iii) a polypeptide which is at least 90%, 95%, 96%, 97%, 98%, 99%, or more, identical to the fragment of (i) or (ii), or (iv) the fragment of (i) or (ii) with 1 to about 20, 1 to about 10, 1 to about 5, or 1 to about 3, conserved amino acid
15 changes.

In other embodiments, the nucleic acid encodes SIRT1 variants comprising amino acids residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1. In another embodiment, the nucleic acid encodes SIRT1 variants comprising a
20 fragment of SEQ ID NO:1 consisting essentially of, or consisting of, amino acid residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1.

In another embodiment, the invention provides a nucleic acid that encodes a SIRT1 variant that is not activatable by resveratrol or other sirtuin activating
25 compounds that bind to the same location on SIRT1 as resveratrol. Such variants may be useful, for example, to identify sirtuin activating compounds that activate SIRT1 by a different mechanism of action. In an exemplary embodiment, the unactivatable SIRT1 variant comprises at least of fragment of SEQ ID NO:1, with the proviso that the variant excludes amino acid residues 183-225 of SEQ ID NO:1.

30 In another embodiment, the nucleic acid comprises, consists essentially of, or consists of, a nucleic acid sequence selected from the group consisting of: (i) a fragment of SEQ ID NO: 2 having a 5' end that falls between nucleic acid residues

418 to 578, 445 to 505, 459 to 550, 459 to 505, 505 to 550 of SEQ ID NO: 2 and a 3' end that falls between nucleic acid residues 1987 to 2110, 1987 to 2011, or 1987 to 2242 of SEQ ID NO:1; (ii) a nucleic acid comprising nucleotide residues 547-1992, 421-2241, 547-2115, 547-2172, 463-1992, 463-2241, 490-1992, 490-2241, 514-1992, 655-1992, 448-2010, or 508-2010 of SEQ ID NO: 2; (iii) a nucleic acid that is at least 90%, 95%, 96%, 97%, 98%, 99%, or more, identical to the nucleic acid of (i) or (ii); or (iv) a nucleic acid that hybridizes under stringent conditions to the nucleic acid of (i) or (ii). In exemplary embodiments, the nucleic acid is a fragment of a full length sirtuin protein. In exemplary embodiments, the nucleic acid encodes a sirtuin variant polypeptide that has a higher expression level than the equivalent wild-type sirtuin, has deacetylase activity that is substantially equivalent to the deacetylase activity of the equivalent wild-type sirtuin, and which has deacetylase activity that may be activated by at least 2-fold in the presence of a sirtuin activating compound.

In some embodiments, the nucleic acid encoding the sirtuin variant may be a naked DNA molecule, or it may be a component of a plasmid, a cosmid, a phagemid, an artificial chromosome, a virus particle or virus-like particle, a liposome, or any similar or equivalent vector which effectively acts to introduce the sirtuin variant nucleotide sequence into the cell. Furthermore, the sirtuin variant nucleic acid advantageously is operably linked to at least one element such as an enhancer, a promoter, or a polyadenylation site that serves to promote the de novo intracellular expression of the encoded sirtuin variant. In another embodiment, the nucleic acid is an isolated or purified nucleic acid.

Nucleic acids which differ from the nucleic acids of the invention due to degeneracy in the genetic code are also within the scope of the invention. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the variants of the invention will exist. One skilled in the art will appreciate that these variations in one or more nucleotides (from less than 1% up to about 3 or 5% or possibly more

of the nucleotides) of the nucleic acids encoding a sirtuin variant of the invention may exist among a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this invention.

- 5 Bias in codon choice within genes in a single species appears related to the level of expression of the protein encoded by that gene. Accordingly, the invention encompasses nucleic acid sequences which have been optimized for improved expression in a host cell by altering the frequency of codon usage in the nucleic acid sequence to approach the frequency of preferred codon usage of the host cell.
- 10 Due to codon degeneracy, it is possible to optimize the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleotide sequence that encodes a sirtuin variant amino acid sequence as set forth herein. In certain embodiments, the expression level of a sirtuin variant is determined with respect to the corresponding full length
- 15 sirtuin protein wherein both the variant and the full length protein have been similarly codon optimized.

Nucleic acids within the scope of the invention may also contain linker sequences, modified restriction endonuclease sites and other sequences useful for molecular cloning, expression or purification of such recombinant variants.

- 20 A nucleic acid encoding a variant of the invention may be obtained from mRNA or genomic DNA from any organism in accordance with protocols described herein, as well as those generally known to those skilled in the art. A cDNA encoding a variant of the invention, for example, may be obtained by isolating total mRNA from an organism, e.g. a bacteria, virus, mammal, etc. Double
- 25 stranded cDNAs may then be prepared from the total mRNA, and subsequently inserted into a suitable plasmid or bacteriophage vector using any one of a number of known techniques. A gene encoding a variant of the invention may also be cloned using established polymerase chain reaction techniques in accordance with the nucleotide sequence information provided herein.

- 30 In another embodiment, a nucleic acid encoding a sirtuin variant is provided in an expression vector such that the nucleotide sequence encoding the sirtuin variant is operably linked to at least one regulatory sequence. It should be

understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of variant desired to be expressed. The vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic
5 markers, should be considered.

The subject nucleic acids may be used to cause expression and over-expression of a sirtuin variant in cells propagated in culture, e.g. to produce proteins or polypeptides, including fusion proteins or polypeptides.

10 4. SIRT1 Recombinant Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding sirtuin variants, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has
15 been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA into which additional DNA segments can be incorporated. Another type of vector is a viral vector, wherein additional DNA segments can be incorporated into the viral genome, or a portion thereof. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g.,
20 bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors
25 are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors
30 (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

Recombinant expression vectors may comprise a nucleic acid encoding a sirtuin variant in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell, those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences), and those that direct inducible expression upon exposure to an external factor such as a small molecule, temperature, etc. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce sirtuin variant polypeptides, including fusion polypeptides, encoded by nucleic acids as described herein.

The recombinant expression vectors of the invention can be designed for expression of a sirtuin variant in prokaryotic or eukaryotic cells. For example, the sirtuin variant can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus or carboxy terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. In certain embodiments, the expression level or activity of a sirtuin variant which is a fusion protein is compared to the expression level or activity of the corresponding wild-type sirtuin protein, wherein both proteins are fused to the same polypeptide sequence at either the N-terminus and/or the C-terminus.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann et al., (1988) *Gene* 69: 301-315) and pET 11d (Studier et al., *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al.,

(1992) *Nucleic Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

5 In another embodiment, the sirtuin variant is expressed using a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., (1987) *EMBO J* 6: 229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30: 933-943), pJRY88 (Schultz et al., (1987) *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

10 Alternatively, the sirtuin variant can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith et al. (1983) *Mol Cell Biol* 3: 2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170: 31-39).

15 In yet another embodiment, sirtuin variants may be expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329: 840) and pMT2PC (Kaufman et al. (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For
20 example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, e.g., Chapters 16 and 17 of Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring
25 Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of a sirtuin variant preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of
30 suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev* 1: 268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43: 235-275), in particular promoters of T cell

receptors (Winoto and Baltimore (1989) EMBO J 8: 729-733) and immunoglobulins (Banerji et al. (1983) Cell 33: 729-740; Queen and Baltimore (1983) Cell 33: 741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) PNAS 86: 5473-5477), pancreas-specific promoters (Edlund et al. (1985) Science 230: 912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss (1990) Science 249: 374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) Genes Dev 3: 537-546).

Also provided are host cells comprising a sirtuin variant polypeptide, host cells comprising a nucleic acid encoding a sirtuin variant polypeptide, host cells comprising an expression vector comprising a nucleic acid sequence that encodes a sirtuin variant, and host cells expressing a sirtuin variant from a nucleic acid. The host cell may be any prokaryotic or eukaryotic cell. For example, a sirtuin variant may be expressed in bacterial cells, such as E. coli, insect cells (baculovirus), yeast, or mammalian cells including, for example, human cells. In those instances when the host cell is human, the cell may be in a live subject or may be isolated from a subject, e.g., in a cell culture, tissue sample, cell suspension, etc. Other suitable host cells are known to those skilled in the art. Additionally, the host cell may be supplemented with tRNA molecules not typically found in the host so as to optimize expression of the polypeptide. Other methods suitable for maximizing expression of the polypeptide will be known to those in the art.

In another variation, production of sirtuin variant polypeptides may be achieved using *in vitro* translation systems. *In vitro* translation systems are, generally, a translation system which is a cell-free extract containing at least the minimum elements necessary for translation of an RNA molecule into a protein. An *in vitro* translation system typically comprises at least ribosomes, tRNAs, initiator methionyl-tRNA^{Met}, proteins or complexes involved in translation, e.g., eIF2, eIF3, the cap-binding (CB) complex, comprising the cap-binding protein (CBP) and eukaryotic initiation factor 4F (eIF4F). A variety of *in vitro* translation systems are

well known in the art and include commercially available kits. Examples of *in vitro* translation systems include eukaryotic lysates, such as rabbit reticulocyte lysates, rabbit oocyte lysates, human cell lysates, insect cell lysates and wheat germ extracts. Lysates are commercially available from manufacturers such as Promega Corp., Madison, Wis.; Stratagene, La Jolla, Calif.; Amersham, Arlington Heights, Ill.; and GIBCO/BRL, Grand Island, N.Y. *In vitro* translation systems typically comprise macromolecules, such as enzymes, translation, initiation and elongation factors, chemical reagents, and ribosomes. In addition, an *in vitro* transcription system may be used. Such systems typically comprise at least an RNA polymerase holoenzyme, ribonucleotides and any necessary transcription initiation, elongation and termination factors. *In vitro* transcription and translation may be coupled in a one-pot reaction to produce proteins from one or more isolated DNAs.

When expression of sirtuin variant having an N-terminal deletion is desired, i.e. the sirtuin variant is a truncation mutant of a full length sirtuin, it may be necessary to add a start codon (ATG) to the oligonucleotide fragment containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position may be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from *E. coli* (Ben-Bassat et al., (1987) *J. Bacteriol.* 169:751-757) and *Salmonella typhimurium* and its *in vitro* activity has been demonstrated on recombinant proteins (Miller et al., (1987) *PNAS USA* 84:2718-1722). Therefore, removal of an N-terminal methionine, if desired, may be achieved either *in vivo* by expressing such recombinant polypeptides in a host which produces MAP (e.g., *E. coli* or CM89 or *S. cerevisiae*), or *in vitro* by use of purified MAP (e.g., procedure of Miller et al.).

Coding sequences for a sirtuin variant may be incorporated as a part of a fusion gene including a nucleotide sequence encoding a different polypeptide. The present invention contemplates a nucleic acid comprising a nucleic acid encoding a sirtuin variant and at least one heterologous sequence encoding a heterologous peptide linked in frame to the nucleotide sequence of the sirtuin variant so as to encode a fusion protein comprising the heterologous polypeptide. The heterologous polypeptide may be fused to (a) the C-terminus of the polypeptide encoded by the nucleic acid of the invention, (b) the N-terminus of the polypeptide, or (c) the C-

terminus and the N-terminus of the polypeptide. In certain instances, the heterologous sequence encodes a polypeptide permitting the detection, isolation, solubilization, transcytosis and/or stabilization of the sirtuin variant polypeptide to which it is fused. In still other embodiments, the heterologous sequence encodes a polypeptide selected from the group consisting of a polyHis tag, myc, HA, GST, protein A, protein G, calmodulin-binding peptide, thioredoxin, maltose-binding protein, poly arginine, poly His-Asp, FLAG, a portion of an immunoglobulin protein, and a transcytosis peptide.

Fusion proteins may facilitate the expression and/or purification of proteins. For example, a sirtuin variant polypeptide may be generated as a glutathione-S-transferase (GST) fusion protein. Such GST fusion proteins may be used to simplify purification of a sirtuin variant polypeptide, such as through the use of glutathione-derivatized matrices (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al., (N.Y.: John Wiley & Sons, 1991)). In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant protein, may allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence may then be subsequently removed by treatment with enterokinase to provide the purified protein (e.g., see Hochuli et al., (1987) J. Chromatography 411: 177; and Janknecht et al., PNAS USA 88:8972).

Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene may be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments may be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which may subsequently be annealed to generate a chimeric gene sequence (see, for example,

Current Protocols in Molecular Biology, eds. Ausubel et al., John Wiley & Sons: 1992).

5. Transgenic Animals

5 Also provided herein are non-human transgenic animals comprising a sirtuin variant as disclosed herein. In an exemplary embodiment, a non-human transgenic animal comprising a SIRT1 variant is provided. A cell transfected with a nucleic acid encoding a sirtuin variant, or a sirtuin variant transfected cell, can be used to produce nonhuman transgenic animals. In one embodiment, a sirtuin variant-
10 transfected cell is a fertilized oocyte or an embryonic stem cell into which a sirtuin variant protein-coding sequence has been introduced. Such cells can then be used to create non-human transgenic animals in which exogenous sirtuin variant sequences have been introduced into the animal's genome or homologous recombinant animals in which endogenous sirtuin protein sequences have been altered. Such animals are
15 useful for studying the function and/or activity of the sirtuin proteins and for identifying and/or evaluating modulators of sirtuin protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal include a transgene. Other examples of transgenic animals include non-human
20 primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is stably integrated into the genome of a cell from which a transgenic animal develops, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal,
25 more preferably a mouse, in which an endogenous SIRT1 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a sirtuin
30 variant protein-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection or retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Sirtuin variant nucleic acids can be

introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human sirtuin variant, such as a mouse sirtuin variant, can be used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene.

- 5 A tissue-specific regulatory sequence(s) can be operably linked to the sirtuin variant transgene to direct expression of the sirtuin variant to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and
- 10 Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the sirtuin variant transgene in its genome and/or expression of sirtuin variant mRNA in tissues or cells of the animals. A
- 15 transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a sirtuin variant can further be bred to other transgenic animals carrying other transgenes.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene.

- 20 One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, e.g., Lakso et al. (1992) PNAS 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) Science 251: 181-185). If a cre/loxP recombinase system is used to regulate expression of
- 25 the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

- 30 Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmot et al. (1997) Nature 385: 810-813. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be

isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

Methods for generating transgenic animals are additionally discussed in "Gene Transfer Methods: Introducing DNA Into Living Cells and Organisms" P. A. Norton and L. F. Steel, Eaton Publishing, 2000; and in "Transgenesis Techniques: Principles and Protocols", 2nd ed., A. R. Clarke, Humana Press, 2002.

In one aspect, a transgenic non-human mammal is provided, a majority of whose cells harbor a transgene comprising a nucleic acid that encodes a sirtuin variant. Preferably, the sirtuin variant is a SIRT1 variant. In one embodiment, the majority of cells which harbor a transgene have increased sirtuin deacetylation activity in respect to cells which do not harbor a transgene.

In certain embodiments, the life span of the transgenic non-human mammal is increased with respect to a nontransgenic mammal of the same species.

6. Assays

Also provided herein are assays for measuring deacetylase activity using the sirtuin variants described herein as well as assays for identifying compounds or agents that modulate sirtuin activity. The assays may be used to identify compounds that either activate sirtuin deacetylase activity or compounds that inhibit sirtuin deacetylase activity. Assays may be conducted in a cell based or cell free format. The assays may be conducted under conditions which permit deacetylation of a substrate by the sirtuin variant. In certain embodiments, the assays are conducted in the presence of NAD⁺. When screening assays are conducted *in vitro*, it may be desirable to contact a cell with a candidate sirtuin modulating compound identified in the *in vitro* screen to determine if the candidate test compound increases the life span of the cell.

Assay methods may involve, for example, contacting at least one acetylated sirtuin substrate with a sirtuin variant polypeptide and determining the level of acetylation of the sirtuin substrate. The assays may further include addition of a test agent to the assay in order to determine if the test agent modulates deacetylation of the substrate by the sirtuin variant as compared to a control (e.g., an assay without the test agent, an assay in the presence of an agent having known sirtuin modulating activity, an assay in the presence of an agent having no sirtuin modulating activity, or a value in a database).

The sirtuin variants described herein may be used in association with various types of deacetylation assays to determine sirtuin activity and/or to identify compounds that modulate sirtuin activity. For example, the sirtuin variants may be used in association with a fluorescence based assay such as the assay commercially available from Biomol, e.g., the SIRT1 Fluorimetric Drug Discovery Kit (AK-555), SIRT2 Fluorimetric Drug Discovery Kit (AK-556), or SIRT3 Fluorimetric Drug Discovery Kit (AK-557) (Biomol International, Plymouth Meeting, PA). Other assay formats that may be used in association with the sirtuin variants described herein include a nicotinamide release assay (Kaeberlein et al., J. Biol. Chem. 280(17): 17038 (2005)), a FRET assay (Marcotte et al., Anal. Biochem. 332: 90 (2004)), and a C¹⁴ NAD boron resin binding assay (McDonagh et al., Methods 36: 346 (2005)). Yet other assay formats that may be used in conjunction with the sirtuin variants described herein include radioimmunoassays (RIA), scintillation proximity assays, HPLC based assays, and reporter gene assays (e.g., for transcription factor targets). In such assays, a sirtuin variant polypeptide may be substituted for the sirtuin protein used in the referenced assays. In other embodiments, the sirtuin variants described herein may be used in association with a fluorescence polarization assay. Examples of fluorescence polarization assays are described herein and are also described in PCT Publication No. WO 2006/094239. In other embodiments, the sirtuin variants described herein may be used in association with mass spectrometry based assays. Examples of mass spectrometry based assays are described herein and are also described in PCT Application No. PCT/US06/046021.

When fluorescence polarization is used as the read out for determining acetylation level, the sirtuin substrate may comprise a fluorophore and a high molecular weight group or bulky group. The high molecular weight or bulky group is separated from the fluorophore by at least one lysine residue. When the lysine residue is in the non-acetylated state, the sirtuin substrate is susceptible to cleavage at or near the lysine residue by a cleavage reagent, such as a protease. When the lysine residue is in the acetylated state, the sirtuin substrate is resistant to cleavage and remains intact upon contact with a cleavage reagent. Upon cleavage, the fluorophore is separated from the high molecular weight or bulky group thereby increasing the fluorescent polarization value of the sample. Exemplary cleavage reagents for use in accordance with the methods described herein include chemical and enzymatic reagents. In an exemplary embodiment, the cleavage reagent is a protease, such as, for example, a protease that cleaves at or near a lysine residue. Exemplary proteases included, for example, lysylendopeptidase, endoproteinase, Lys-C, plasmin, calpain, or trypsin.

One advantage of FP is that it can be utilized in homogeneous assays and therefore is useful for high throughput screening (HTS) assays. FP is also amenable to performing assays in real-time, directly in solution and without the need for an immobilized phase. Polarization values can be measured repeatedly both before and after the addition of reagents since measuring the samples is rapid and does not destroy the sample.

In other embodiments, the methods described herein for determining sirtuin activity and/or for identifying a compound that modulates sirtuin activity utilize mass spectrometry for determining the level of acetylation of a sirtuin substrate. The presence of an acetyl group on a polypeptide may be determined by a +42 Da molecular weight shift (per acetyl group) as compared to the unmodified polypeptide. Mass spectrometry (or simply MS) encompasses any spectrometric technique or process in which molecules are ionized and separated and/or analyzed based on their respective molecular weights. Thus, mass spectrometry and MS encompass any type of ionization method, including without limitation electrospray ionization (ESI), atmospheric-pressure chemical ionization (APCI) and other forms of atmospheric pressure ionization (API), and laser irradiation. Mass spectrometers

may be combined with separation methods such as gas chromatography (GC) and liquid chromatography (LC). GC or LC separates the components in a mixture, and the components are then individually introduced into the mass spectrometer; such techniques are generally called GC/MS and LC/MS, respectively.

5 In various embodiments, the assays described herein utilize a sirtuin substrate pool that comprises a plurality of copies of one or more sirtuin substrate polypeptides. In an exemplary embodiment, a sirtuin substrate pool comprises a plurality of copies of the same polypeptide substrate. Such sirtuin substrate pools may comprise the sirtuin substrate free floating in solution or attached to a solid
10 surface such as a plate, bead, filter, etc. Combinations of free floating and anchored sirtuin substrate molecules may also be used in accordance with the methods described herein. Substrates suitable for use in accordance with the methods described herein may be based on any polypeptide that can be deacetylated by a sirtuin protein, such as, for example, p53 or histones. Exemplary substrates include,
15 for example, the Fluor de Lys-SIRT1 substrate from BIOMOL (Plymouth Meeting, PA). Other suitable substrates, including for FP and mass spec based assays include, for example, Ac-EE-K(biotin)-GQSTSSHSK(Ac)NleSTEG-K(MR121)-EE-NH₂ (SEQ ID NO: 3) and Ac-EE-K(biotin)-GQSTSSHSK(Ac)NleSTEG-K(5TMR)-EE-NH₂ (SEQ ID NO: 4) wherein K(biotin) is a biotinylated lysine residue, K(Ac) is an acetylated lysine residue, Nle is norleucine, K(MR121) is a lysine residue modified by an MR121 fluorophore (excitation 635 nm/emission 680 nm), and K(5TMR) is a lysine residue modified by a 5TMR fluorophore (excitation 540 nm/emission 580 nm). The sequence of the peptide substrates are based on p53 with several modifications. In particular, all arginine and leucine residues other than the
20 acetylated lysine residues are replaced with serine so that the peptides are not susceptible to trypsin cleavage in the absence of deacetylation. In addition, the methionine residues naturally present in the sequences are replaced with the norleucine because the methionine may be susceptible to oxidation during synthesis and purification.

30 In certain embodiments, the sirtuin assays described herein may be carried out in a single reaction vessel without the need to remove reagents from the reaction mixture (e.g., a homogenous assay). In various embodiments, the components of the

reactions described herein may be added sequentially or simultaneously. For example, it is possible to add a cleavage reagent concurrently with, or subsequent to, exposure of the sirtuin substrate to the sirtuin deacetylase.

In certain embodiments, the invention provides a method for identifying a
5 compound that modulates the activity of a sirtuin deacetylase. The methods may involve comparing the activity of a sirtuin protein in the presence of a test compound as compared to a control. The control may be the activity of a sirtuin protein in a control reaction or a value in a database. A control reaction may simply be a duplicate reaction in which the test compound is not included. Alternatively,
10 the control reaction may be a duplicate reaction in the presence of a compound having a known effect on the sirtuin protein activity (e.g., an activator, an inhibitor, or a compound having no effect on enzyme activity).

In certain embodiments, the invention provides methods for screening for compounds that modulate activity of a sirtuin deacetylases. In certain embodiments,
15 the methods described herein may be used to identify a test compound that decreases or increases sirtuin activity by at least about 10%, 25%, 50%, 75%, 80%, 90%, or 100%, or more, relative to the activity in the absence of the test compound.

Test compounds to be tested for activity in the assays described herein can include proteins (including post-translationally modified proteins), peptides
20 (including chemically or enzymatically modified peptides), or small molecules (including carbohydrates, steroids, lipids, anions or cations, drugs, small organic molecules, oligonucleotides, antibodies, and genes encoding proteins of the agents or antisense molecules), including libraries of compounds. The test compounds can be naturally occurring (e.g., found in nature or isolated from nature) or can be non-
25 naturally occurring (e.g., synthetic, chemically synthesized or man-made).

If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead
30 one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide

oligomer, or small molecule libraries of compounds. See Lam, Anticancer Drug Des. 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt et al., Proc. Natl. Acad. Sci. U.S.A. 90, 6909, 1993; Erb et al. Proc. Natl. Acad. Sci. U.S.A. 91, 11422, 1994; Zuckermann et al., J Med Chem. 37,2678, 1994; Cho et al., Science 261, 1303, 1993; Carell et al., Angew. Chem. Int. Ed Engl. 33, 2059, 1994; Carell et al., Angew. Chem. Int. Ed. Engl. 33, 2061; Gallop et al., J. Med Chem. 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, BioTechniques 13, 412-421, 1992), or on beads (Lam, Nature 354, 82-84, 1991), chips (Fodor, Nature 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Pat. No. 5,223,409), plasmids (Cull et al., Proc. Natl. Acad. Sci. U.S.A. 89, 1865-1869, 1992), or phage (Scott & Smith, Science 249, 386-390, 1990; Devlin, Science 249, 404-406, 1990); Cwirla et al., Proc. Natl. Acad. Sci. 97, 6378-6382, 1990; Felici, J. Mol. Biol. 222, 301-310, 1991; and Ladner, U.S. Pat. No. 5,223,409).

Test compounds can be screened for the ability to modulate acetyltransferase or deacetylase activity using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, free format assays, or assays that have no physical barrier between samples, can be used. Assays involving free formats are described, for example, in Jayawickreme et al., Proc. Natl. Acad. Sci. U.S.A. 19, 1614-18 (1994); Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995); and Salmon et al., Molecular Diversity 2, 57-63 (1996). Another high throughput screening method is described in Beutel et al., U.S. Pat. No. 5,976,813.

Compounds that activate or inhibit the acetyltransferase or deacetylase activity, which can be selected according to the method for screening of the present

invention, are useful as candidate compounds for antimicrobial substances, anti-cancer agents, and a variety of other uses. For example, compounds that activate a sirtuin deacetylase protein may be useful for increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, chemotherapeutic induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. In other embodiments, sirtuin deacetylase inhibitors may be useful for a variety of therapeutic applications including, for example, increasing cellular sensitivity to stress, increasing apoptosis, treatment of cancer, stimulation of appetite, and/or stimulation of weight gain, etc.

7. Therapeutic Treatment

In certain embodiments, the invention provides methods of treating a variety of diseases and disorders in which an increase in sirtuin activity is desirable. The methods involve increasing sirtuin activity by administering to a subject in need thereof a sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant. It is to be understood that the term sirtuin variant therapeutic agent encompasses a single sirtuin variant polypeptide, a combination of two or more sirtuin variant polypeptides, a single nucleic acid encoding a sirtuin variant polypeptide, a combination nucleic acids encoding two or more sirtuin variant polypeptides, as well as combinations of sirtuin variant polypeptides and nucleic acids encoding sirtuin variant polypeptides. Exemplary sirtuin variant polypeptides and nucleic acids encoding sirtuin variant polypeptides are described in detail above. Examples of diseases and disorders that would benefit from an increase in sirtuin activity include, for example, aging or stress, diabetes, obesity, neurodegenerative diseases, chemotherapeutic induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. Exemplary diseases and disorders that would benefit from an increase in sirtuin activity are discussed in more detail below.

The therapeutic methods described herein involve increasing sirtuin activity in a subject in need thereof using a sirtuin variant therapeutic agent as described herein. The methods may involve, for example, administering to a subject a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide. When
5 administering a sirtuin variant polypeptide to a subject, the sirtuin variant polypeptide may be modified, for example, to increase its stability or to facilitate cellular uptake. Such modifications are described further herein. In an exemplary embodiment, a sirtuin variant polypeptide is fused to a transcytosis peptide (such as, for example, TAT or a fragment thereof) to facilitate cellular uptake, to an Fc
10 domain to increase serum stability, or both. Alternatively, the methods described herein may utilize a nucleic acid encoding a sirtuin variant polypeptide. Sirtuin variant therapeutic agents may be administered systemically to a subject or may be administered locally to a desired location in the body as appropriate for the specific disease or disorder being treated. When using a nucleic acid therapeutic, the
15 methods also contemplate *ex vivo* gene therapy methods, e.g., wherein cells are removed from a subject, transfected *in vitro* with the desired nucleic acid, followed by reintroduction of the modified cells into the same or a different subject. Methods of administering protein and nucleic acid therapeutics are described further herein. In exemplary embodiments, the methods involve administering a human SIRT1
20 variant polypeptide or a nucleic acid encoding a SIRT1 variant polypeptide to a human patient in need of increased SIRT1 activity.

In exemplary embodiments, administration of the sirtuin variant therapeutic agent results in an increase in sirtuin activity in the subject. Such an increase in sirtuin activity may be achieved, for example, by increasing the number of active
25 sirtuin proteins in the cell using the sirtuin variant therapeutic agent, by introducing a sirtuin variant therapeutic agent into a cell which does not typically have sirtuin activity, or by introducing a sirtuin variant therapeutic agent into a cell having regulated sirtuin activity thereby producing a cell having sirtuin activity on a constitutive basis. When using a nucleic acid therapeutic it may be desirable to have
30 expression of the sirtuin variant under the control of a constitutive promoter and/or a promoter that produces a higher level of sirtuin expression than the native sirtuin promoter. In other embodiments, a sirtuin variant may be under the control of a

tissue or cell specific promoter or under the control of the native sirtuin promoter so that sirtuin variant expression is confined to a specific cell or tissue type or limited to a native level of protein expression. A nucleic acid encoding a sirtuin variant that does not contain its own promoter may also be introduced into the genome of a host cell so that expression is controlled by a promoter that is endogenous to the host cell.

In certain embodiments, a sirtuin variant therapeutic agent may be administered alone or in combination with other compounds. In one embodiment, a mixture of two or more sirtuin variant therapeutic agents may be administered to a subject in need thereof. Such combination may comprise, for example, a mixture of two different sirtuin variant polypeptides, a sirtuin variant polypeptide and a nucleic acid encoding the same or a different sirtuin variant polypeptide, or a combination of two nucleic acids encoding different sirtuin variant polypeptides. In another embodiment, sirtuin variant therapeutic agents may be used in combination with one or more of the following compounds: resveratrol, butein, fisetin, piceatannol, or quercetin. In an exemplary embodiment, sirtuin variant therapeutic agents may be administered in combination with nicotinic acid. In yet another embodiment, one or more sirtuin variant therapeutic agents (e.g., sirtuin variant polypeptides or nucleic acids encoding sirtuin variant polypeptides) may be used as a therapeutic in combination with one or more additional therapeutic agents for the treatment or prevention of various diseases, including, for example, cancer, diabetes, neurodegenerative diseases, cardiovascular disease, blood clotting, inflammation, flushing, obesity, ageing, stress, etc. In various embodiments, combination therapies comprising a sirtuin variant therapeutic agent may refer to (1) pharmaceutical compositions that comprise one or more sirtuin variant therapeutic agents in combination with one or more additional therapeutic agents (e.g., one or more additional therapeutic agents described herein); and (2) co-administration of one or more sirtuin variant therapeutic agents with one or more additional therapeutic agents wherein the sirtuin variant therapeutic agent and the additional therapeutic agent have not been formulated in the same compositions (but may be present within the same kit or package, such as a blister pack or other multi-chamber package; connected, separately sealed containers (e.g., foil pouches) that can be separated by the user; or a kit where the sirtuin variant therapeutic agent(s) and other additional

therapeutic agent(s) are in separate vessels). When using separate formulations, sirtuin variant therapeutic agents may be administered at the same, intermittent, staggered, prior to, subsequent to, or combinations thereof, with the administration of another therapeutic agent.

- 5 Methods contemplated by the present invention include methods of extending the life span of a eukaryotic cell. In one embodiment, a method of extending the life span of a eukaryotic cell comprises introducing into the cell a sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide, such as, for example, a SIRT1 variant.
- 10 Expression of the sirtuin variant in the cell may lead to, for example, inhibition or delay of population doubling and/or differentiation of the cell. The eukaryotic cell so transformed may be in an *in vitro* cell culture, or it may be in an *ex vivo* tissue or organ sample, or it may exist *in vivo* as a constituent of a living organism. In exemplary embodiments, the transfected or transformed cell may be a vertebrate
- 15 cell, a mammalian cell, or a human cell.

Other methods contemplated by the invention include methods for mimicking the effects of calorie restriction in a eukaryotic cell comprising introducing into the eukaryotic cell a sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide.

20 ***Aging/Stress***

- In one embodiment, the invention provides a method extending the lifespan of a cell, extending the proliferative capacity of a cell, slowing ageing of a cell, promoting the survival of a cell, delaying cellular senescence in a cell, mimicking the effects of calorie restriction, increasing the resistance of a cell to stress, or
- 25 preventing apoptosis of a cell, by introducing into the cell a sirtuin variant therapeutic agent.

- The methods described herein may be used to increase the amount of time that cells, particularly primary cells (i.e., cells obtained from an organism, e.g., a human), may be kept alive in a cell culture. Embryonic stem (ES) cells and
- 30 pluripotent cells, and cells differentiated therefrom, may also be modified with a sirtuin variant therapeutic agent to keep the cells, or progeny thereof, in culture for

longer periods of time. Such cells can also be used for transplantation into a subject, e.g., after *ex vivo* modification.

In one embodiment, cells that are intended to be preserved for long periods of time may be modified with a sirtuin variant therapeutic agent. The cells may be
5 in suspension (e.g., blood cells, serum, biological growth media, etc.) or in tissues or organs. For example, blood collected from an individual for purposes of transfusion may be modified with sirtuin variant therapeutic agent to preserve the blood cells for longer periods of time. Additionally, blood to be used for forensic purposes may also be preserved using a sirtuin variant therapeutic agent. Other
10 cells that may be treated to extend their lifespan or protect against apoptosis include cells for consumption, e.g., cells from non-human mammals (such as meat) or plant cells (such as vegetables).

Sirtuin variant therapeutic agents may also be introduced into cells during developmental and growth phases in mammals, plants, insects or microorganisms,
15 in order to, e.g., alter, retard or accelerate the developmental and/or growth process.

In another embodiment, sirtuin variant therapeutic agents may be used to treat cells useful for transplantation or cell therapy, including, for example, solid tissue grafts, organ transplants, cell suspensions, stem cells, bone marrow cells, etc. The cells or tissue may be an autograft, an allograft, a syngraft or a xenograft. The
20 cells or tissue may be treated with a sirtuin variant therapeutic agent prior to administration/implantation, concurrently with administration/implantation, and/or post administration/implantation into a subject. The cells or tissue may be treated prior to removal of the cells from the donor individual, *ex vivo* after removal of the cells or tissue from the donor individual, or post implantation into the recipient.
25 For example, the donor or recipient individual may be treated systemically with a sirtuin variant therapeutic agent or may have a subset of cells/tissue treated locally with a sirtuin variant therapeutic agent. In certain embodiments, the cells or tissue (or donor/recipient individuals) may additionally be treated with another therapeutic agent useful for prolonging graft survival, such as, for example, an
30 immunosuppressive agent, a cytokine, an angiogenic factor, etc.

In yet other embodiments, cells may be modified with a sirtuin variant therapeutic agent *in vivo*, e.g., to increase their lifespan or prevent apoptosis. For

example, skin can be protected from aging (e.g., developing wrinkles, loss of elasticity, etc.) by treating skin or epithelial cells with a sirtuin variant therapeutic agent. In an exemplary embodiment, skin is contacted with a pharmaceutical or cosmetic composition comprising a sirtuin variant therapeutic agent. Exemplary

5 skin afflictions or skin conditions that may be treated in accordance with the methods described herein include disorders or diseases associated with or caused by inflammation, sun damage or natural aging. For example, the compositions find utility in the prevention or treatment of contact dermatitis (including irritant contact dermatitis and allergic contact dermatitis), atopic dermatitis (also known as allergic

10 eczema), actinic keratosis, keratinization disorders (including eczema), epidermolysis bullosa diseases (including penfigus), exfoliative dermatitis, seborrheic dermatitis, erythemas (including erythema multiforme and erythema nodosum), damage caused by the sun or other light sources, discoid lupus erythematosus, dermatomyositis, psoriasis, skin cancer and the effects of natural

15 aging. In another embodiment, sirtuin variant therapeutic agents may be used for the treatment of wounds and/or burns to promote healing, including, for example, first-, second- or third-degree burns and/or a thermal, chemical or electrical burns. The formulations may be administered topically, to the skin or mucosal tissue, as an ointment, lotion, cream, microemulsion, gel, solution or the like, as further

20 described herein, within the context of a dosing regimen effective to bring about the desired result.

Topical formulations comprising one or more sirtuin variant therapeutic agents may also be used as preventive, e.g., chemopreventive, compositions. When used in a chemopreventive method, susceptible skin is treated prior to any visible

25 condition in a particular individual.

Sirtuin variant therapeutic agents may be delivered locally or systemically to a subject. In one embodiment, a sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide, is delivered locally to a tissue or organ of a subject by injection, topical formulation,

30 etc.

In another embodiment, a sirtuin variant therapeutic agent may be used for treating or preventing a disease or condition induced or exacerbated by cellular

senescence in a subject; methods for decreasing the rate of senescence of a subject, e.g., after onset of senescence; methods for extending the lifespan of a subject; methods for treating or preventing a disease or condition relating to lifespan; methods for treating or preventing a disease or condition relating to the
5 proliferative capacity of cells; and methods for treating or preventing a disease or condition resulting from cell damage or death. In certain embodiments, the method does not act by decreasing the rate of occurrence of diseases that shorten the lifespan of a subject. In certain embodiments, a method does not act by reducing the lethality caused by a disease, such as cancer.

10 In yet another embodiment, a sirtuin variant therapeutic agent may be administered to a subject in order to generally increase the lifespan of its cells and to protect its cells against stress and/or against apoptosis. It is believed that treating a subject with a sirtuin variant therapeutic agent described herein is similar to subjecting the subject to hormesis, i.e., mild stress that is beneficial to organisms
15 and may extend their lifespan.

Sirtuin variant therapeutic agents may be administered to a subject to prevent aging and aging-related consequences or diseases, such as stroke, heart disease, heart failure, arthritis, high blood pressure, and Alzheimer's disease. Other conditions that can be treated include ocular disorders, e.g., associated with the
20 aging of the eye, such as cataracts, glaucoma, and macular degeneration. Sirtuin variant therapeutic agents can also be administered to subjects for treatment of diseases, e.g., chronic diseases, associated with cell death, in order to protect the cells from cell death. Exemplary diseases include those associated with neural cell death, neuronal dysfunction, or muscular cell death or dysfunction, such as
25 Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and muscular dystrophy; AIDS; fulminant hepatitis; diseases linked to degeneration of the brain, such as Creutzfeld-Jakob disease, retinitis pigmentosa and cerebellar degeneration; myelodysplasia such as aplastic anemia; ischemic diseases such as myocardial infarction and stroke; hepatic diseases such as
30 alcoholic hepatitis, hepatitis B and hepatitis C; joint-diseases such as osteoarthritis; atherosclerosis; alopecia; damage to the skin due to UV light; lichen planus;

atrophy of the skin; cataract; and graft rejections. Cell death can also be caused by surgery, drug therapy, chemical exposure or radiation exposure.

Sirtuin variant therapeutic agents can also be administered to a subject suffering from an acute disease, e.g., damage to an organ or tissue, e.g., a subject
5 suffering from stroke or myocardial infarction or a subject suffering from a spinal cord injury. Sirtuin variant therapeutic agents may also be used to repair an alcoholic's liver.

Cardiovascular Disease

In another embodiment, the invention provides a method for treating and/or
10 preventing a cardiovascular disease by administering to a subject in need thereof a sirtuin variant therapeutic agent.

Cardiovascular diseases that can be treated or prevented by increasing sirtuin activity, e.g. using a sirtuin variant therapeutic agent, include cardiomyopathy or myocarditis; such as idiopathic cardiomyopathy, metabolic cardiomyopathy,
15 alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy. Also treatable or preventable using sirtuin variant therapeutic agents are atheromatous disorders of the major blood vessels (macrovascular disease) such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the
20 femoral arteries, and the popliteal arteries. Other vascular diseases that can be treated or prevented include those related to platelet aggregation, the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems. The sirtuin variant therapeutic agents may also be
25 used for increasing HDL levels in plasma of an individual.

Yet other disorders that may be treated with sirtuin variant therapeutic agents include restenosis, e.g., following coronary intervention, and disorders relating to an abnormal level of high density and low density cholesterol.

In one embodiment, a sirtuin variant therapeutic agent may be administered
30 as part of a combination therapeutic with another cardiovascular agent including, for example, an anti-arrhythmic agent, an antihypertensive agent, a calcium channel blocker, a cardioplegic solution, a cardiotonic agent, a fibrinolytic agent, a

sclerosing solution, a vasoconstrictor agent, a vasodilator agent, a nitric oxide donor, a potassium channel blocker, a sodium channel blocker, statins, or a natriuretic agent.

In one embodiment, a sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or nucleic acid encoding a sirtuin variant polypeptide, may be administered as part of a combination therapeutic with an anti-arrhythmia agent. Anti-arrhythmia agents are often organized into four main groups according to their mechanism of action: type I, sodium channel blockade; type II, beta-adrenergic blockade; type III, repolarization prolongation; and type IV, calcium channel blockade. Type I anti-arrhythmic agents include lidocaine, moricizine, mexiletine, tocainide, procainamide, encainide, flecanide, tocainide, phenytoin, propafenone, quinidine, disopyramide, and flecainide. Type II anti-arrhythmic agents include propranolol and esmolol. Type III includes agents that act by prolonging the duration of the action potential, such as amiodarone, artilide, bretylium, clofilium, isobutilide, sotalol, azimilide, dofetilide, dronedarone, ersentilide, ibutilide, tedisamil, and trecetilide. Type IV anti-arrhythmic agents include verapamil, diltiazem, digitalis, adenosine, nickel chloride, and magnesium ions.

In another embodiment, a sirtuin variant therapeutic agent may be administered as part of a combination therapeutic with another cardiovascular agent. Examples of cardiovascular agents include vasodilators, for example, hydralazine; angiotensin converting enzyme inhibitors, for example, captopril; anti-anginal agents, for example, isosorbide nitrate, glyceryl trinitrate and pentaerythritol tetranitrate; anti-arrhythmic agents, for example, quinidine, procainamide and lignocaine; cardioglycosides, for example, digoxin and digitoxin; calcium antagonists, for example, verapamil and nifedipine; diuretics, such as thiazides and related compounds, for example, bendrofluazide, chlorothiazide, chlorothalidone, hydrochlorothiazide and other diuretics, for example, furosemide and triamterene, and sedatives, for example, nitrazepam, flurazepam and diazepam.

Other exemplary cardiovascular agents include, for example, a cyclooxygenase inhibitor such as aspirin or indomethacin, a platelet aggregation inhibitor such as clopidogrel, ticlopidine or aspirin, fibrinogen antagonists or a diuretic such as chlorothiazide, hydrochlorothiazide, flumethiazide,

hydroflumethiazide, bendroflumethiazide, methylchlorthiazide, trichloromethiazide, polythiazide or benzthiazide as well as ethacrynic acid, tricyclic, chlorthalidone, furosemide, musolimine, bumetanide, triamterene, amiloride and spironolactone and salts of such compounds, angiotensin converting enzyme inhibitors such as
5 captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazopril, delapril, pentopril, quinapril, ramipril, lisinopril, and salts of such compounds, angiotensin II antagonists such as losartan, irbesartan or valsartan, thrombolytic agents such as tissue plasminogen activator (tPA), recombinant tPA, streptokinase, urokinase, prourokinase, and anisoylated plasminogen streptokinase activator complex
10 (APSAC, Eminase, Beecham Laboratories), or animal salivary gland plasminogen activators, calcium channel blocking agents such as verapamil, nifedipine or diltiazem, thromboxane receptor antagonists such as ifetroban, prostacyclin mimetics, or phosphodiesterase inhibitors. Such combination products if formulated as a fixed dose employ the compounds of this invention within the dose range
15 described above and the other pharmaceutically active agent within its approved dose range.

Yet other exemplary cardiovascular agents include, for example, vasodilators, e.g., bencyclane, cinnarizine, citicoline, cyclandelate, cyclonate, ebumamonine, phenoxethyl, flunarizine, ibudilast, ifenprodil, lomerizine, naphlole,
20 nikamate, nosergoline, nimodipine, papaverine, pentifylline, nifedipine, vincamin, vinpocetine, vichizyl, pentoxifylline, prostacyclin derivatives (such as prostaglandin E1 and prostaglandin I2), an endothelin receptor blocking drug (such as bosentan), diltiazem, nicorandil, and nitroglycerin. Examples of the cerebral protecting drug include radical scavengers (such as edaravone, vitamin E, and vitamin C), glutamate
25 antagonists, AMPA antagonists, kainate antagonists, NMDA antagonists, GABA agonists, growth factors, opioid antagonists, phosphatidylcholine precursors, serotonin agonists, $\text{Na}^+/\text{Ca}^{2+}$ channel inhibitory drugs, and K^+ channel opening drugs. Examples of the brain metabolic stimulants include amantadine, tiapride, and gamma-aminobutyric acid. Examples of the anticoagulant include heparins (such as
30 heparin sodium, heparin potassium, dalteparin sodium, dalteparin calcium, heparin calcium, parnaparin sodium, reviparin sodium, and danaparoid sodium), warfarin, enoxaparin, argatroban, batroxobin, and sodium citrate. Examples of the antiplatelet

drug include ticlopidine hydrochloride, dipyridamole, cilostazol, ethyl icosapentate, sarpogrelate hydrochloride, dilazep hydrochloride, trapidil, a nonsteroidal antiinflammatory agent (such as aspirin), beraprostsodium, iloprost, and indobufene. Examples of the thrombolytic drug include urokinase, tissue-type plasminogen

5 activators (such as alteplase, tisokinase, nateplase, pamiteplase, monteplase, and rateplase), and nasaruplase. Examples of the antihypertensive drug include angiotensin converting enzyme inhibitors (such as captopril, alacepril, lisinopril, imidapril, quinapril, temocapril, delapril, benazepril, cilazapril, trandolapril, enalapril, ceronapril, fosinopril, imadapril, mobertpril, perindopril, ramipril,

10 spirapril, and randolapril), angiotensin II antagonists (such as losartan, candesartan, valsartan, eprosartan, and irbesartan), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendilin, galopamil, mibefradil, prenylamine,

15 semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline), β -adrenaline receptor blocking drugs (propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol,

20 betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, xybenolol, and esmolol),

25 α -receptor blocking drugs (such as amosulalol, prazosin, terazosin, doxazosin, bunazosin, urapidil, phentolamine, arotinolol, dapiprazole, fenspiride, indoramin, labetalol, naftopidil, nicergoline, tamsulosin, tolazoline, trimazosin, and yohimbine), sympathetic nerve inhibitors (such as clonidine, guanfacine, guanabenz, methyl dopa, and reserpine), hydralazine, todralazine, budralazine, and cadralazine. Examples of

30 the antianginal drug include nitrate drugs (such as amyl nitrite, nitroglycerin, and isosorbide), β -adrenaline receptor blocking drugs (such as propranolol, pindolol,

indanolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevigolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, andxybenolol), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendiline, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline) trimetazidine, dipyridamole, etafenone, dilazep, trapidil, nicorandil, enoxaparin, and aspirin. Examples of the diuretic include thiazide diuretics (such as hydrochlorothiazide, methyclothiazide, trichlormethiazide, benzylhydrochlorothiazide, and penflutizide), loop diuretics (such as furosemide, etacrynic acid, bumetanide, piretanide, azosemide, and torasemide), K^+ sparing diuretics (spironolactone, triamterene, andpotassiumcanrenoate), osmotic diuretics (such as isosorbide, D-mannitol, and glycerin), nonthiazide diuretics (such as meticrane, tripamide, chlorthalidone, and mefruside), and acetazolamide. Examples of the cardiotonic include digitalis formulations (such as digitoxin, digoxin, methyl digoxin, deslanoside, vesnarinone, lanatoside C, and proscillaridin), xanthine formulations (such as aminophylline, choline theophylline, diprophylline, and proxiphylline), catecholamine formulations (such as dopamine, dobutamine, and docarpamine), PDE III inhibitors (such as amrinone, olprinone, and milrinone), denopamine, ubidecarenone, pimobendan, levosimendan, aminoethylsulfonic acid, vesnarinone, carperitide, and colforsin daropate. Examples of the antiarrhythmic drug include ajmaline, pirmenol, procainamide, cibenzoline, disopyramide, quinidine, aprindine, mexiletine, lidocaine, phenyloin, pilsicainide, propafenone, flecainide, atenolol, acebutolol, sotalol, propranolol, metoprolol, pindolol, amiodarone, nifekalant, diltiazem, bepridil, and verapamil. Examples of the antihyperlipidemic drug include

atorvastatin, simvastatin, pravastatin sodium, fluvastatin sodium, clonofibrate, clofibrate, simfibrate, fenofibrate, bezafibrate, colestimide, and colestyramine. Examples of the immunosuppressant include azathioprine, mizoribine, cyclosporine, tacrolimus, gusperimus, and methotrexate.

5 ***Cell Death/Cancer***

Sirtuin variant therapeutic agents may be administered to subjects who have recently received or are likely to receive a dose of radiation or toxin. In one embodiment, the dose of radiation or toxin is received as part of a work-related or medical procedure, e.g., working in a nuclear power plant, flying an airplane, an X-ray, CAT scan, or the administration of a radioactive dye for medical imaging; in such an embodiment, the sirtuin variant therapeutic agent is administered as a prophylactic measure. In another embodiment, the radiation or toxin exposure is received unintentionally, e.g., as a result of an industrial accident, habitation in a location of natural radiation, terrorist act, or act of war involving radioactive or toxic material. In such a case, the sirtuin variant therapeutic agent is preferably administered as soon as possible after the exposure to inhibit apoptosis and the subsequent development of acute radiation syndrome.

Sirtuin variant therapeutic agents may also be used for treating and/or preventing cancer. Calorie restriction has been linked to a reduction in the incidence of age-related disorders including cancer (see e.g., Bordone and Guarente, Nat. Rev. Mol. Cell Biol. (2005 epub); Guarente and Picard, Cell 120: 473-82 (2005); Berrigan, et al., Carcinogenesis 23: 817-822 (2002); and Heilbronn and Ravussin, Am. J. Clin. Nutr. 78: 361-369 (2003)). Additionally, the Sir2 protein from yeast has been shown to be required for lifespan extension by glucose restriction (see e.g., Lin et al., Science 289: 2126-2128 (2000); Anderson et al., Nature 423: 181-185 (2003)), a yeast model for calorie restriction. Accordingly, an increase in the level and/or activity of a sirtuin protein may be useful for treating and/or preventing the incidence of age-related disorders, such as, for example, cancer. Exemplary cancers that may be treated by increasing sirtuin activity, e.g., using a sirtuin variant therapeutic agent such as a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide, are those of the brain and kidney; hormone-dependent cancers including breast, prostate, testicular, and

ovarian cancers; lymphomas, and leukemias. In cancers associated with solid tumors, a sirtuin variant therapeutic agent may be administered directly into the tumor. Cancer of blood cells, e.g., leukemia, can be treated by administering a sirtuin variant therapeutic agent into the blood stream or into the bone marrow.

- 5 Benign cell growth can also be treated, e.g., warts. Other diseases that can be treated include autoimmune diseases, e.g., systemic lupus erythematosus, scleroderma, and arthritis, in which autoimmune cells should be removed. Viral infections such as herpes, HIV, adenovirus, and HTLV-1 associated malignant and benign disorders can also be treated by administration of a sirtuin variant
10 therapeutic agent. Alternatively, cells can be obtained from a subject, treated *ex vivo* to remove certain undesirable cells, e.g., cancer cells, and administered back to the same or a different subject.

Neuronal Diseases/Disorders

- In certain aspects, sirtuin variant therapeutic agents can be used to treat
15 patients suffering from neurodegenerative diseases, and traumatic or mechanical injury to the central nervous system (CNS), spinal cord or peripheral nervous system (PNS). Neurodegenerative disease typically involves reductions in the mass and volume of the human brain, which may be due to the atrophy and/or death of brain cells, which are far more profound than those in a healthy person that are attributable
20 to aging. Neurodegenerative diseases can evolve gradually, after a long period of normal brain function, due to progressive degeneration (e.g., nerve cell dysfunction and death) of specific brain regions. Alternatively, neurodegenerative diseases can have a quick onset, such as those associated with trauma or toxins. The actual onset of brain degeneration may precede clinical expression by many years. Examples of
25 neurodegenerative diseases include, but are not limited to, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), diffuse Lewy body disease, chorea-acanthocytosis, primary lateral sclerosis, ocular diseases (ocular neuritis), chemotherapy-induced neuropathies (e.g., from vincristine, paclitaxel, bortezomib),
30 diabetes-induced neuropathies and Friedreich's ataxia. These disorders, and others described below, may be treated by increasing sirtuin activity in a subject, e.g., using a sirtuin variant therapeutic agent as described herein.

AD is a chronic, incurable, and unstoppable CNS disorder that occurs gradually, resulting in memory loss, unusual behavior, personality changes, and a decline in thinking abilities. These losses are related to the death of specific types of brain cells and the breakdown of connections and their supporting network (e.g. glial cells) between them. AD has been described as childhood development in reverse. In most people with AD, symptoms appear after the age 60. The earliest symptoms include loss of recent memory, faulty judgment, and changes in personality. Later in the disease, those with AD may forget how to do simple tasks like washing their hands. Eventually people with AD lose all reasoning abilities and become dependent on other people for their everyday care. Finally, the disease becomes so debilitating that patients are bedridden and typically develop coexisting illnesses.

PD is a chronic, incurable, and unstoppable CNS disorder that occurs gradually and results in uncontrolled body movements, rigidity, tremor, and dyskinesia. These motor system problems are related to the death of brain cells in an area of the brain that produces dopamine, a chemical that helps control muscle activity. In most people with PD, symptoms appear after age 50. The initial symptoms of PD are a pronounced tremor affecting the extremities, notably in the hands or lips. Subsequent characteristic symptoms of PD are stiffness or slowness of movement, a shuffling walk, stooped posture, and impaired balance. There are wide ranging secondary symptoms such as memory loss, dementia, depression, emotional changes, swallowing difficulties, abnormal speech, sexual dysfunction, and bladder and bowel problems. These symptoms will begin to interfere with routine activities, such as holding a fork or reading a newspaper. Finally, people with PD become so profoundly disabled that they are bedridden.

ALS (motor neuron disease) is a chronic, incurable, and unstoppable CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles. In ALS, the motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, the command to move never reaches the muscles. Most people who get ALS are between 40 and 70 years old. The first motor neurons that weaken are those controlling the arms or legs. Those with ALS may have trouble walking, they may drop things, fall, slur their speech, and laugh or cry uncontrollably. Eventually the

muscles in the limbs begin to atrophy from disuse. This muscle weakness will become debilitating and a person will need a wheel chair or become unable to function out of bed.

The causes of these neurological diseases have remained largely unknown.

5 They are conventionally defined as distinct diseases, yet clearly show extraordinary similarities in basic processes and commonly demonstrate overlapping symptoms far greater than would be expected by chance alone. Current disease definitions fail to properly deal with the issue of overlap and a new classification of the neurodegenerative disorders has been called for.

10 HD is another neurodegenerative disease resulting from genetically programmed degeneration of neurons in certain areas of the brain. This degeneration causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance. HD is a familial disease, passed from parent to child through a dominant mutation in the wild-type gene. Some early symptoms of HD are mood
15 swings, depression, irritability or trouble driving, learning new things, remembering a fact, or making a decision. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult and the patient may have difficulty feeding himself or herself and swallowing.

Tay-Sachs disease and Sandhoff disease are glycolipid storage diseases
20 caused by the lack of lysosomal β -hexosaminidase (Gravel et al., in *The Metabolic Basis of Inherited Disease*, eds. Scriver et al., McGraw-Hill, New York, pp. 2839-2879, 1995). In both disorders, GM2 ganglioside and related glycolipidssubstrates for β -hexosaminidase accumulate in the nervous system and trigger acute neurodegeneration. In the most severe forms, the onset of symptoms begins in early
25 infancy. A precipitous neurodegenerative course then ensues, with affected infants exhibiting motor dysfunction, seizure, visual loss, and deafness. Death usually occurs by 2-5 years of age. Neuronal loss through an apoptotic mechanism has been demonstrated (Huang et al., *Hum. Mol. Genet.* 6: 1879-1885, 1997).

HIV-1 also induces neurological disease. Shi et al. (*J. Clin. Invest.* 98: 1979-
30 1990, 1996) examined apoptosis induced by HIV-1 infection of the CNS in an *in vitro* model and in brain tissue from AIDS patients, and found that HIV-1 infection

of primary brain cultures induced apoptosis in neurons and astrocytes *in vitro*. Apoptosis of neurons and astrocytes was also detected in brain tissue from 10/11 AIDS patients, including 5/5 patients with HIV-1 dementia and 4/5 nondemented patients.

5 There are four main peripheral neuropathies associated with HIV, namely sensory neuropathy, AIDP/CIPD, drug-induced neuropathy and CMV-related. The most common type of neuropathy associated with AIDS is distal symmetrical polyneuropathy (DSPN). This syndrome is a result of nerve degeneration and is characterized by numbness and a sensation of pins and needles. DSPN causes few
10 serious abnormalities and mostly results in numbness or tingling of the feet and slowed reflexes at the ankles. It generally occurs with more severe immunosuppression and is steadily progressive. Treatment with tricyclic antidepressants relieves symptoms but does not affect the underlying nerve damage.

 A less frequent, but more severe type of neuropathy is known as acute or
15 chronic inflammatory demyelinating polyneuropathy (AIDP/CIDP). In AIDP/CIDP there is damage to the fatty membrane covering the nerve impulses. This kind of neuropathy involves inflammation and resembles the muscle deterioration often identified with long-term use of AZT. It can be the first manifestation of HIV infection, where the patient may not complain of pain, but fails to respond to
20 standard reflex tests. This kind of neuropathy may be associated with seroconversion, in which case it can sometimes resolve spontaneously. It can serve as a sign of HIV infection and indicate that it might be time to consider antiviral therapy. AIDP/CIDP may be auto-immune in origin.

 Drug-induced, or toxic, neuropathies can be very painful. Antiviral drugs
25 commonly cause peripheral neuropathy, as do other drugs e.g. vincristine, dilantin (an anti-seizure medication), high-dose vitamins, isoniazid, and folic acid antagonists. Peripheral neuropathy is often used in clinical trials for antivirals as a dose-limiting side effect, which means that more drugs should not be administered. Additionally, the use of such drugs can exacerbate otherwise minor neuropathies.
30 Usually, these drug-induced neuropathies are reversible with the discontinuation of the drug.

CMV causes several neurological syndromes in AIDS, including encephalitis, myelitis, and polyradiculopathy.

Neuronal loss is also a salient feature of prion diseases, such as Creutzfeldt-Jakob disease in human, BSE in cattle (mad cow disease), Scrapie Disease in sheep
5 and goats, and feline spongiform encephalopathy (FSE) in cats. Increasing sirtuin activity, e.g., using a sirtuin variant therapeutic agent may be useful for treating or preventing neuronal loss due to these prior diseases.

In another embodiment, sirtuin variant therapeutic agents may be used to treat or prevent any disease or disorder involving axonopathy. Distal axonopathy is
10 a type of peripheral neuropathy that results from some metabolic or toxic derangement of peripheral nervous system (PNS) neurons. It is the most common response of nerves to metabolic or toxic disturbances, and as such may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes such as malnutrition and alcoholism, or the effects of toxins or drugs. The most common
15 cause of distal axonopathy is diabetes, and the most common distal axonopathy is diabetic neuropathy. The most distal portions of axons are usually the first to degenerate, and axonal atrophy advances slowly towards the nerve's cell body. If the noxious stimulus is removed, regeneration is possible, though prognosis decreases depending on the duration and severity of the stimulus. Those with distal
20 axonopathies usually present with symmetrical glove-stocking sensori-motor disturbances. Deep tendon reflexes and autonomic nervous system (ANS) functions are also lost or diminished in affected areas.

Diabetic neuropathies are neuropathic disorders that are associated with diabetes mellitus. These conditions usually result from diabetic microvascular injury
25 involving small blood vessels that supply nerves (vasa nervorum). Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuritis multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. Clinical manifestations of diabetic neuropathy include, for example, sensorimotor
30 polyneuropathy such as numbness, sensory loss, dysesthesia and nighttime pain; autonomic neuropathy such as delayed gastric emptying or gastroparesis; and cranial

neuropathy such as oculomotor (3rd) neuropathies or Mononeuropathies of the thoracic or lumbar spinal nerves.

Peripheral neuropathy is the medical term for damage to nerves of the peripheral nervous system, which may be caused either by diseases of the nerve or
5 from the side-effects of systemic illness. Peripheral neuropathies vary in their presentation and origin, and may affect the nerve or the neuromuscular junction. Major causes of peripheral neuropathy include seizures, nutritional deficiencies, and HIV, though diabetes is the most likely cause. Mechanical pressure from staying in one position for too long, a tumor, intraneural hemorrhage, exposing the body to
10 extreme conditions such as radiation, cold temperatures, or toxic substances can also cause peripheral neuropathy.

In an exemplary embodiment, a sirtuin variant therapeutic agent may be used to treat or prevent multiple sclerosis (MS), including relapsing MS and monosymptomatic MS, and other demyelinating conditions, such as, for example, chronic
15 inflammatory demyelinating polyneuropathy (CIDP), or symptoms associated therewith.

MS is a chronic, often disabling disease of the central nervous system. Various and converging lines of evidence point to the possibility that the disease is caused by a disturbance in the immune function, although the cause of this
20 disturbance has not been established. This disturbance permits cells of the immune system to "attack" myelin, the fat containing insulating sheath that surrounds the nerve axons located in the central nervous system ("CNS"). When myelin is damaged, electrical pulses cannot travel quickly or normally along nerve fiber pathways in the brain and spinal cord. This results in disruption of normal electrical
25 conductivity within the axons, fatigue and disturbances of vision, strength, coordination, balance, sensation, and bladder and bowel function.

As such, MS is now a common and well-known neurological disorder that is characterized by episodic patches of inflammation and demyelination which can occur anywhere in the CNS. However, almost always without any involvement of
30 the peripheral nerves associated therewith. Demyelination produces a situation analogous to that resulting from cracks or tears in an insulator surrounding an electrical cord. That is, when the insulating sheath is disrupted, the circuit is "short

circuited" and the electrical apparatus associated therewith will function intermittently or not at all. Such loss of myelin surrounding nerve fibers results in short circuits in nerves traversing the brain and the spinal cord that thereby result in symptoms of MS. It is further found that such demyelination occurs in patches, as opposed to along the entire CNS. In addition, such demyelination may be intermittent. Therefore, such plaques are disseminated in both time and space.

It is believed that the pathogenesis involves a local disruption of the blood brain barrier which causes a localized immune and inflammatory response, with consequent damage to myelin and hence to neurons.

Clinically, MS exists in both sexes and can occur at any age. However, its most common presentation is in the relatively young adult, often with a single focal lesion such as a damage of the optic nerve, an area of anesthesia (loss of sensation), or paraesthesia (localized loss of feeling), or muscular weakness. In addition, vertigo, double vision, localized pain, incontinence, and pain in the arms and legs may occur upon flexing of the neck, as well as a large variety of less common symptoms.

An initial attack of MS is often transient, and it may be weeks, months, or years before a further attack occurs. Some individuals may enjoy a stable, relatively event free condition for a great number of years, while other less fortunate ones may experience a continual downhill course ending in complete paralysis. There is, most commonly, a series of remission and relapses, in which each relapse leaves a patient somewhat worse than before. Relapses may be triggered by stressful events, viral infections or toxins. Therein, elevated body temperature, i.e., a fever, will make the condition worse, or as a reduction of temperature by, for example, a cold bath, may make the condition better.

In yet another embodiment, sirtuin variant therapeutic agents may be used to treat trauma to the nerves, including, trauma due to disease, injury (including surgical intervention), or environmental trauma (e.g., neurotoxins, alcoholism, etc.).

Sirtuin variant therapeutic agents may also be useful to prevent, treat, and alleviate symptoms of various PNS disorders, such as the ones described below. The PNS is composed of the nerves that lead to or branch off from the spinal cord and CNS. The peripheral nerves handle a diverse array of functions in the body, including sensory, motor, and autonomic functions. When an individual has a

peripheral neuropathy, nerves (either afferent or efferent) of the PNS have been damaged. Nerve damage can arise from a number of causes, such as disease, physical injury, poisoning, or malnutrition. Depending on the cause of damage, the nerve cell axon, its protective myelin sheath, or both may be injured or destroyed.

5 The term “peripheral neuropathy” encompasses a wide range of disorders in which the nerves outside of the brain and spinal cord—peripheral nerves—have been damaged. Peripheral neuropathy may also be referred to as peripheral neuritis, or if many nerves are involved, the terms polyneuropathy or polyneuritis may be used.

10 Peripheral neuropathy is a widespread disorder, and there are many underlying causes. Some of these causes are common, such as diabetes, and others are extremely rare, such as acrylamide poisoning and certain inherited disorders. The most common worldwide cause of peripheral neuropathy is leprosy. Leprosy is caused by the bacterium *Mycobacterium leprae*, which attacks the peripheral nerves
15 of affected people.

Leprosy is extremely rare in the United States, where diabetes is the most commonly known cause of peripheral neuropathy. It has been estimated that more than 17 million people in the United States and Europe have diabetes-related polyneuropathy. Many neuropathies are idiopathic; no known cause can be found.
20 The most common of the inherited peripheral neuropathies in the United States is Charcot-Marie-Tooth disease, which affects approximately 125,000 persons.

Another of the better known peripheral neuropathies is Guillain-Barré syndrome, which arises from complications associated with viral illnesses, such as cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus (HIV), or
25 bacterial infection, including *Campylobacter jejuni* and Lyme disease. The worldwide incidence rate is approximately 1.7 cases per 100,000 people annually. Other well-known causes of peripheral neuropathies include chronic alcoholism, infection of the varicella-zoster virus, botulism, and poliomyelitis. Peripheral neuropathy may develop as a primary symptom, or it may be due to another disease.
30 For example, peripheral neuropathy is only one symptom of diseases such as amyloid neuropathy, certain cancers, or inherited neurologic disorders. Such diseases may affect the PNS and the CNS, as well as other body tissues.

Other PNS diseases treatable with sirtuin variant therapeutic agents include: Brachial Plexus Neuropathies (diseases of the cervical and first thoracic roots, nerve trunks, cords, and peripheral nerve components of the brachial plexus. Clinical manifestations include regional pain, paresthesia; muscle weakness, and decreased sensation in the upper extremity. These disorders may be associated with trauma, including birth injuries; thoracic outlet syndrome; neoplasms, neuritis, radiotherapy; and other conditions. See Adams et al., Principles of Neurology, 6th ed, pp1351-2); Diabetic Neuropathies (peripheral, autonomic, and cranial nerve disorders that are associated with diabetes mellitus). These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum). Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuritis multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy (see Adams et al., Principles of Neurology, 6th ed, p1325); mononeuropathies (disease or trauma involving a single peripheral nerve in isolation, or out of proportion to evidence of diffuse peripheral nerve dysfunction). Mononeuritis multiplex refers to a condition characterized by multiple isolated nerve injuries. Mononeuropathies may result from a wide variety of causes, including ischemia; traumatic injury; compression; connective tissue diseases; cumulative trauma disorders; and other conditions; Neuralgia (intense or aching pain that occurs along the course or distribution of a peripheral or cranial nerve); Peripheral Nervous System Neoplasms (neoplasms which arise from peripheral nerve tissue). This includes neurofibromas; Schwannomas; granular cell tumors; and malignant peripheral nerve sheath tumors (see DeVita Jr et al., Cancer: Principles and Practice of Oncology, 5th ed, pp1750-1); and Nerve Compression Syndromes (mechanical compression of nerves or nerve roots from internal or external causes. These may result in a conduction block to nerve impulses, due to, for example, myelin sheath dysfunction, or axonal loss. The nerve and nerve sheath injuries may be caused by ischemia; inflammation; or a direct mechanical effect; Neuritis (a general term indicating inflammation of a peripheral or cranial nerve). Clinical manifestation may include pain; paresthesias; paresis; or hyperesthesia; Polyneuropathies (diseases of multiple peripheral nerves). The various forms are categorized by the type of

nerve affected (e.g., sensory, motor, or autonomic), by the distribution of nerve injury (e.g., distal vs. proximal), by nerve component primarily affected (e.g., demyelinating vs. axonal), by etiology, or by pattern of inheritance.

In another embodiment, sirtuin variant therapeutic agents may be used to
5 treat or prevent chemotherapeutic induced neuropathy. The sirtuin variant therapeutic agent may be administered prior to administration of the chemotherapeutic agent, concurrently with administration of the chemotherapeutic drug, and/or after initiation of administration of the chemotherapeutic drug. If the sirtuin variant therapeutic agent is administered after the initiation of administration
10 of the chemotherapeutic drug, it is desirable that the sirtuin variant therapeutic agent be administered prior to, or at the first signs, of chemotherapeutic induced neuropathy.

Chemotherapy drugs can damage any part of the nervous system. Encephalopathy and myelopathy are fortunately very rare. Damage to peripheral
15 nerves is much more common and can be a side effect of treatment experienced by people with cancers, such as lymphoma. Most of the neuropathy affects sensory rather than motor nerves. Thus, the common symptoms are tingling, numbness or a loss of balance. The longest nerves in the body seem to be most sensitive hence the fact that most patients will report numbness or pins and needles in their hands and
20 feet.

The chemotherapy drugs which are most commonly associated with neuropathy, are the Vinca alkaloids (anti-cancer drugs originally derived from a member of the periwinkle - the Vinca plant genus) and a platinum- containing drug called Cisplatin. The Vinca alkaloids include the drugs vinblastine, vincristine and
25 vindesine. Many combination chemotherapy treatments for lymphoma for example CHOP and CVP contain vincristine, which is the drug known to cause this problem most frequently. Indeed, it is the risk of neuropathy that limits the dose of vincristine that can be administered.

Studies that have been performed have shown that most patients will lose
30 some reflexes in their legs as a result of treatment with vincristine and many will experience some degree of tingling (paresthesia) in their fingers and toes. The neuropathy does not usually manifest itself right at the start of the treatment but

generally comes on over a period of a few weeks. It is not essential to stop the drug at the first onset of symptoms, but if the neuropathy progresses this may be necessary. It is very important that patients should report such symptoms to their doctors, as the nerve damage is largely reversible if the drug is discontinued. Most
5 doctors will often reduce the dose of vincristine or switch to another form of Vinca alkaloid such as vinblastine or vindesine if the symptoms are mild. Occasionally, the nerves supplying the bowel are affected causing abdominal pain and constipation.

In another embodiment, a sirtuin variant therapeutic agent may be used to treat or prevent a polyglutamine disease. Huntington's Disease (HD) and
10 Spinocerebellar ataxia type 1 (SCA1) are just two examples of a class of genetic diseases caused by dynamic mutations involving the expansion of triplet sequence repeats. In reference to this common mechanism, these disorders are called trinucleotide repeat diseases. At least 14 such diseases are known to affect human beings. Nine of them, including SCA1 and Huntington's disease, have CAG as the
15 repeated sequence (see Table 2 below). Since CAG codes for an amino acid called glutamine, these nine trinucleotide repeat disorders are collectively known as polyglutamine diseases.

Although the genes involved in different polyglutamine diseases have little in common, the disorders they cause follow a strikingly similar course. Each disease
20 is characterized by a progressive degeneration of a distinct group of nerve cells. The major symptoms of these diseases are similar, although not identical, and usually affect people in midlife. Given the similarities in symptoms, the polyglutamine diseases are hypothesized to progress via common cellular mechanisms. In recent years, scientists have made great strides in unraveling what the mechanisms are.

25 Above a certain threshold, the greater the number of glutamine repeats in a protein, the earlier the onset of disease and the more severe the symptoms. This suggests that abnormally long glutamine tracts render their host protein toxic to nerve cells.

To test this hypothesis, scientists have generated genetically engineered mice
30 expressing proteins with long polyglutamine tracts. Regardless of whether the mice express full-length proteins or only those portions of the proteins containing the polyglutamine tracts, they develop symptoms of polyglutamine diseases. This

suggests that a long polyglutamine tract by itself is damaging to cells and does not have to be part of a functional protein to cause its damage.

For example, it is thought that the symptoms of SCA1 are not directly caused by the loss of normal ataxin-1 function but rather by the interaction between ataxin-1 and another protein called LANP. LANP is needed for nerve cells to communicate with one another and thus for their survival. When the mutant ataxin-1 protein accumulates inside nerve cells, it “traps” the LANP protein, interfering with its normal function. After a while, the absence of LANP function appears to cause nerve cells to malfunction.

Table 2. Summary of Polyglutamine Diseases.

Disease	Gene name	Chromosomal location	Pattern of inheritance	Protein	Normal repeat length	Disease repeat length
Spinobulbar muscular atrophy (Kennedy disease)	<i>AR</i>	Xq13–21	X-linked recessive	androgen receptor (AR)	9–36	38–62
Huntington's disease	<i>HD</i>	4p16.3	autosomal dominant	huntingtin	6–35	36–121
Dentatorubral-pallidoluysian atrophy (Haw River syndrome)	<i>DRPLA</i>	12p13.31	autosomal dominant	atrophin-1	6–35	49–88
Spinocerebellar ataxia type 1	<i>SCA1</i>	6p23	autosomal dominant	ataxin-1	6–44	39–82
Spinocerebellar ataxia type 2	<i>SCA2</i>	12q24.1	autosomal dominant	ataxin-2	15–31	36–63
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	<i>SCA3</i>	14q32.1	autosomal dominant	ataxin-3	12–40	55–84
Spinocerebellar ataxia type 6	<i>SCA6</i>	19p13	autosomal dominant	α_1 -voltage-dependent calcium channel subunit	4–18	21–33
Spinocerebellar ataxia type 7	<i>SCA7</i>	3p12–13	autosomal dominant	ataxin-7	4–35	37–306
Spinocerebellar ataxia type 17	<i>SCA17</i>	6q27	autosomal dominant	<i>TATA binding protein</i>	25–42	45–63

Many transcription factors have also been found in neuronal inclusions in different diseases. It is possible that these transcription factors interact with the

polyglutamine-containing proteins and then become trapped in the neuronal inclusions. This in turn might keep the transcription factors from turning genes on and off as needed by the cell. Another observation is hypoacetylation of histones in affected cells. This has led to the hypothesis that Class I/II Histone Deacetylase (HDAC I/II) inhibitors, which are known to increase histone acetylation, may be a novel therapy for polyglutamine diseases (see e.g., US Patent Application Publication No. 2004/0142859, entitled "Method of treating neurodegenerative, psychiatric, and other disorders with deacetylase inhibitors").

In yet another embodiment, the invention provides a method for treating or preventing neuropathy related to ischemic injuries or diseases, such as, for example, coronary heart disease (including congestive heart failure and myocardial infarctions), stroke, emphysema, hemorrhagic shock, peripheral vascular disease (upper and lower extremities) and transplant related injuries.

In certain embodiments, the invention provides a method to treat a central nervous system cell to prevent damage in response to a decrease in blood flow to the cell. Typically the severity of damage that may be prevented will depend in large part on the degree of reduction in blood flow to the cell and the duration of the reduction. By way of example, the normal amount of perfusion to brain gray matter in humans is about 60 to 70 mL/100 g of brain tissue/min. Death of central nervous system cells typically occurs when the flow of blood falls below approximately 8-10 mL/100 g of brain tissue/min, while at slightly higher levels (i.e. 20-35 mL/100 g of brain tissue/min) the tissue remains alive but not able to function. In one embodiment, apoptotic or necrotic cell death may be prevented. In still a further embodiment, ischemic-mediated damage, such as cytotoxic edema or central nervous system tissue anoxemia, may be prevented. In each embodiment, the central nervous system cell may be a spinal cell or a brain cell.

Another aspect encompasses administering a sirtuin variant therapeutic agent to a subject to treat a central nervous system ischemic condition. A number of central nervous system ischemic conditions may be treated by increasing sirtuin activity using the sirtuin variant therapeutic agents described herein. In one embodiment, the ischemic condition is a stroke that results in any type of ischemic central nervous system damage, such as apoptotic or necrotic cell death, cytotoxic

edema or central nervous system tissue anoxia. The stroke may impact any area of the brain or be caused by any etiology commonly known to result in the occurrence of a stroke. In one alternative of this embodiment, the stroke is a brain stem stroke. Generally speaking, brain stem strokes strike the brain stem, which control
5 involuntary life-support functions such as breathing, blood pressure, and heartbeat. In another alternative of this embodiment, the stroke is a cerebellar stroke. Typically, cerebellar strokes impact the cerebellum area of the brain, which controls balance and coordination. In still another embodiment, the stroke is an embolic stroke. In general terms, embolic strokes may impact any region of the brain and
10 typically result from the blockage of an artery by a vaso-occlusion. In yet another alternative, the stroke may be a hemorrhagic stroke. Like ischemic strokes, hemorrhagic stroke may impact any region of the brain, and typically result from a ruptured blood vessel characterized by a hemorrhage (bleeding) within or surrounding the brain. In a further embodiment, the stroke is a thrombotic stroke.
15 Typically, thrombotic strokes result from the blockage of a blood vessel by accumulated deposits.

In another embodiment, the ischemic condition may result from a disorder that occurs in a part of the subject's body outside of the central nervous system, but yet still causes a reduction in blood flow to the central nervous system. These
20 disorders may include, but are not limited to a peripheral vascular disorder, a venous thrombosis, a pulmonary embolus, arrhythmia (e.g. atrial fibrillation), a myocardial infarction, a transient ischemic attack, unstable angina, or sickle cell anemia. Moreover, the central nervous system ischemic condition may occur as result of the subject undergoing a surgical procedure. By way of example, the subject may be
25 undergoing heart surgery, lung surgery, spinal surgery, brain surgery, vascular surgery, abdominal surgery, or organ transplantation surgery. The organ transplantation surgery may include heart, lung, pancreas, kidney or liver transplantation surgery. Moreover, the central nervous system ischemic condition may occur as a result of a trauma or injury to a part of the subject's body outside the
30 central nervous system. By way of example, the trauma or injury may cause a degree of bleeding that significantly reduces the total volume of blood in the subject's body.

Because of this reduced total volume, the amount of blood flow to the central

nervous system is concomitantly reduced. By way of further example, the trauma or injury may also result in the formation of a vaso-occlusion that restricts blood flow to the central nervous system.

Of course it is contemplated that the sirtuin variant therapeutic agents may be employed to treat the central nervous system ischemic condition irrespective of the cause of the condition. In one embodiment, the ischemic condition results from a vaso-occlusion. The vaso-occlusion may be any type of occlusion, but is typically a cerebral thrombosis or an embolism. In a further embodiment, the ischemic condition may result from a hemorrhage. The hemorrhage may be any type of hemorrhage, but is generally a cerebral hemorrhage or a subarachnoid hemorrhage. In still another embodiment, the ischemic condition may result from the narrowing of a vessel. Generally speaking, the vessel may narrow as a result of a vasoconstriction such as occurs during vasospasms, or due to arteriosclerosis. In yet another embodiment, the ischemic condition results from an injury to the brain or spinal cord.

In yet another aspect, a sirtuin variant therapeutic agent may be administered to reduce infarct size of the ischemic core following a central nervous system ischemic condition. Moreover, a sirtuin variant therapeutic agent may also be beneficially administered to reduce the size of the ischemic penumbra or transitional zone following a central nervous system ischemic condition.

In one embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of neurodegenerative disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin variant therapeutic agents, e.g., one or more sirtuin variant polypeptides, one or more nucleic acids encoding a sirtuin variant polypeptide, or combinations thereof, and one or more anti-neurodegeneration agents. For example, one or more sirtuin variant therapeutic agents can be combined with an effective amount of one or more of: L-DOPA; a dopamine agonist; an adenosine A2A receptor antagonist; a COMT inhibitor; a MAO inhibitor; an N-NOS inhibitor; a sodium channel antagonist; a selective N-methyl D-aspartate (NMDA) receptor antagonist; an AMPA/kainate receptor antagonist; a calcium channel antagonist; a GABA-A receptor agonist; an acetyl-

choline esterase inhibitor; a matrix metalloprotease inhibitor; a PARP inhibitor; an inhibitor of p38 MAP kinase or c-jun-N-terminal kinases; TPA; NDA antagonists; beta-interferons; growth factors; glutamate inhibitors; and/or as part of a cell therapy.

5 Exemplary N-NOS inhibitors include 4-(6-amino-pyridin-2-yl)-3-methoxyphenol 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,3-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-2,3-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-methyl)piperidinyloxy)-2,3-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethoxy]-3-methoxy-phenyl}-pyridin-2-yl-amine, 6-[3-methoxy-4-[2-(4-phenethyl-piperazin-1-yl)-ethoxy]-phenyl]-pyridin-2-yl-amine, 6-[3-methoxy-4-[2-(4-methyl-piperazin-1-yl)-ethoxy]-phenyl]-pyridin-2-yl-amine, 6-[4-[2-(4-dimethylamino-piperidin-1-yl)-ethoxy]-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-3-ethoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-3-ethoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropyl-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclopropyl-phenol 6-[2-cyclopropyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 3-[3-(6-amino-pyridin-2-yl)-4-cyclopropyl-phenoxy]-pyrrolidine-1-carboxylic acid tert-butyl ester 6-[2-cyclopropyl-4-(1-methylpyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclobutyl-phenol 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(1-methylpyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclopentyl-phenol 6-[2-cyclopentyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopentyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 3-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-pyrrolidine-1-carboxylic acid tert butyl ester 6-[4-(1-methylpyrrolidin-3-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 4-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-piperidine-1-carboxylic acid tert butyl ester 6-[2-methoxy-4-(1-methyl-p-

iperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(allyloxy)-2-methoxy-phenyl]-
 pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-methoxy-6-allyl-phenol 12 and 4-(6-
 amino-pyridin-2-yl)-3-methoxy-2-allyl-phenol 13 4-(6-amino-pyridin-2-yl)-3-
 methoxy-6-propyl-phenol 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-5-propyl-
 5 phenyl]-pyridin-yl-amine, 6-[2-isopropyl-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-
 yl-amine, 6-[2-isopropyl-4-(piperidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(1-methyl-azetidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amin-e 6-[2-
 10 isopropyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(2-methyl-2-aza-bicyclo[2.2.1]hept-5-yl-oxy)-phenyl]-p-yridin-2-yl-
 amine, 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-
 {4-[2-(benzyl-methyl-amino)-ethoxy]-2-methoxy-phenyl}-pyridin-2-yl-amine, 6-[2-
 methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 2-(6-amino-
 15 pyridin-2-yl)-5-(2-dimethylamino-ethoxy)-phenol 2-[4-(6-amino-pyridin-2-yl)-3-
 methoxy-phenoxy]-acetamide 6-[4-(2-amino-ethoxy)-2-methoxy-phenyl]-pyridin-2-
 yl-amine, 6-{4-[2-(3,4-dihydro-1h-isoquinolin-2-yl)-ethoxy]-2-methoxy-phenyl}-
 pyridin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethanol 6-
 {2-methoxy-4-[2-(2,2,6,6-tetramethyl-piperidin-1-yl)-ethoxy]-phenyl}-pyridin-2-
 20 yl-amine, 6-{4-[2-(2,5-dimethyl-pyrrolidin-1-yl)-ethoxy]-2-methoxy-phenyl}-
 pyridin-2-yl-amine, 6-{4-[2-(2,5-dimethyl-pyrrolidin-1-yl)-ethoxy]-2-methoxy-
 phenyl}-pyridin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-1-
 (2,2,6,6-tetramethyl-piperidin-1-yl)-ethanone 6-[2-methoxy-4-(1-methyl-pyrrolidin-
 2-yl-methoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-
 25 propoxy-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2-
 propoxy-phenyl}-pyridin-2-yl-amin-e 6-[4-(2-ethoxy-ethoxy)-2-methoxy-phenyl]-
 pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropoxy-phenyl]-pyridin-
 2-yl-amine, 6-[4-(2-ethoxy-ethoxy)-2-isopropoxy-phenyl]-pyridin-2-yl-amine, 6-[2-
 methoxy-4-(3-methyl-butoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-
 30 ethoxy)-2-ethoxy-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-
 ethoxy]-2-ethoxy-phenyl}-pyridin-2-yl-amine, 6-[2-ethoxy-4-(3-methyl-butoxy)-
 phenyl]-pyridin-2-yl-amine, 1-(6-amino-3-aza-bicyclo[3.1.0]hex-3-yl)-2-[4-(6-

amino-pyridin-2-yl)-3-ethoxy-phenoxy]-ethanone 6-[2-ethoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 3-{2-[4-(6-amino-pyridin-2-yl)-3-ethoxy-phenoxy]-ethyl}-3-aza-bicyclo[3.1.0]hex-6-yl-amine, 1-(6-amino-3-aza-bicyclo[3.1.0]hex-3-yl)-2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethanone
 5 3-{2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethyl}-3-aza-bicyclo[3.1.0]hex-6-yl-amine, 6-[2-isopropoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2-isopropoxy-phenyl}-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-2-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[3-allyl-4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-ethoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(2,2,6,6-tetramethyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 3-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-azetidine-1-carboxylic acid tert-butyl ester 6-[4-(azetidin-3-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-azetidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(2-methyl-2-aza-bicyclo[2.2.1]hept-5-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(1-ethyl-piperidin-4-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,6-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(3-piperidin-1-yl-propoxy)-phenyl]-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{2,6-dimethyl-4-[3-(4-methyl-piperazin-1-yl)-

propoxy]-phenyl]-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(2-morpholin-4-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2,6-dimethyl-phenyl}-p-yrudin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3,5-dimethyl-phenoxy]-acetamide 6-[4-(2-amino-ethoxy)-2,6-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2-isopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 2-(2,5-dimethyl-pyrrolidin-1-yl)-6-[2-isopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridine 6-{4-[2-(3,5-dimethyl-piperidin-1-yl)-ethoxy]-2-isopropyl-phenyl}-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropyl-phenyl]-pyridin-2-yl-amine, 6-[2-tert-butyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-tert-butyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-(4-phenethylpiperazin-1-yl)-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopropyl-4-(2-dimethylamino-1-methyl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(allyloxy)-2-cyclobutyl-phenyl]-pyridin-2-yl-amine, 2-allyl-4-(6-amino-pyridin-2-yl)-3-cyclobutyl-phenol and 2-allyl-4-(6-amino-pyridin-2-yl)-5-cyclobutyl-phenol 4-(6-amino-pyridin-2-yl)-3-cyclobutyl-2-propyl-phenol 4-(6-amino-pyridin-2-yl)-3-cyclobutyl-2-propyl-phenol 6-[2-cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[cyclobutyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 2-(4-benzyloxy-5-hydroxy-2-methoxy-phenyl)-6-(2,5-dimethyl-pyrrol-1-yl)-p-yridine 6-[4-(2-dimethylamino-ethoxy)-5-ethoxy-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-ethyl-2-methoxy-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[5-ethyl-2-methoxy-4-(piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2,5-dimethoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-5-ethyl-2-methoxy-phenyl]-pyridin-2-yl-amine.

Exemplary NMDA receptor antagonist include (+)-(1S, 2S)-1-(4-hydroxy-phenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, (1S, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, (3R, 4S)-3-(4-(4-fluorophenyl)-4-hydroxypiperidin-1-yl)-chroman-4,7-diol, (1R*, 2R*)-1-(4-hydroxy-3-methylphenyl)-2-(4-(4-fluoro-phenyl)-4-hydroxypiperidin-1-yl)-propan-1-ol-mesylate or a pharmaceutically acceptable acid addition salt thereof.

Exemplary dopamine agonist include ropininole; L-dopa decarboxylase inhibitors such as carbidopa or benserazide, bromocriptine, dihydroergocryptine, etisulergine, AF-14, alaptide, pergolide, piribedil; dopamine D1 receptor agonists such as A-68939, A-77636, dihydrexine, and SKF-38393; dopamine D2 receptor agonists such as carbergoline, lisuride, N-0434, naxagolide, PD-118440, pramipexole, quinpirole and ropinirole; dopamine/ β -adrenergic receptor agonists such as DPDMS and dopexamine; dopamine/5-HT uptake inhibitor/5-HT-1A agonists such as roxindole; dopamine/opiate receptor agonists such as NIH-10494; α 2-adrenergic antagonist/dopamine agonists such as terguride; α 2-adrenergic antagonist/dopamine D2 agonists such as ergolines and talipexole; dopamine uptake inhibitors such as GBR-12909, GBR-13069, GYKI-52895, and NS-2141; monoamine oxidase-B inhibitors such as selegiline, N-(2-butyl)-N-methylpropargylamine, N-methyl-N-(2-pentyl)propargylamine, AGN-1133, ergot derivatives, lazabemide, LU-53439, MD-280040 and mofegiline; and COMT inhibitors such as CGP-28014.

Exemplary acetyl cholinesterase inhibitors include donepezil, 1-(2-methyl-1H-benzimidazol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-phenyl-1H-benzimidazol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-ethyl-2-methyl-1H-benzimidazol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-methyl-6-benzothiazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-methyl-6-benzothiazolyl)-3-[1-[(2-methyl-4-thiazolyl)methyl]-4-piperidinyl]-1-propanone; 1-(5-methyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(3,5-dimethyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(benzo[b]thien-2-yl)-3-[1-

(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(benzofuran-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-phenylsulfonyl-6-methyl-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-phenylsulfonyl-5-amino-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-amino-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; and 1-(5-acetylamino-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone. 1-(6-quinolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-indolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-benzthienyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-quinazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-benzoxazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-benzofuranyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-methyl-benzimidazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzimidazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-chloro-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-azaindol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-azabenz[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1H-2-oxo-pyrrolo[2',3',5,6]benzo[b]thieno-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzothiazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methoxy-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methoxy-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-acetylamino-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-acetylamino-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 6-hydroxy-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 5-methyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-methoxy-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-acetamide-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-amino-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(4-morpholinyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 5,7-dihydro-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6H-pyrrolo[4,5-f]-1,2-benzisoxazol-6-one; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisothiazole; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethenyl]-1,2-

benzisoxazole; 6-phenylamino-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(2-thiazolyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(2-oxazolyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-pyrrolidinyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 5,7-dihydro-5,5-dimethyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6H-pyrrolo[4,5-f]-1,2-benzisoxazole-6-one; 6,8-dihydro-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-7H-pyrrolo[5,4-g]-1,2-benzisoxazole-7-one; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-5,6,8-trihydro-7H-isoxazolo[4,5-g]-quinolin-7-one; 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-ylidenyl)methylpiperidine, 1-benzyl-4-((5-methoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-diethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-methnylenedioxy-1-indanon)-2-yl)methylpiperidine, 1-(m-nitrobenzyl)-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-cyclohexymethyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-(m-florobenzyl)-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)propylpiperidine, and 1-benzyl-4-((5-isopropoxy-6-methoxy-1-indanon)-2-yl)methylpiperidine.

Exemplary calcium channel antagonists include diltiazem, omega-conotoxin GVIA, methoxyverapamil, amlodipine, felodipine, lacidipine, and mibefradil.

Exemplary GABA-A receptor modulators include clomethiazole; IDDB; gaboxadol (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol); ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one); fengabine (2-[(butylimino)-(2-chlorophenyl)methyl]-4-chlorophenol); 2-(4-methoxyphenyl)-2,5,6,7,8,9-hexahydro-pyrazolo[4,3-c]cinnolin-3-one; 7-cyclobutyl-6-(2-methyl-2H-1,2,4-triazol-3-ylmethoxy)-3-phenyl-1,2,4-triazolo[4,3-b]pyridazine; (3-fluoro-4-methylphenyl)-N-(1-[(2-methylphenyl)methyl]-benzimidazol-2-yl)methyl-N-pentylcarboxamide; and 3-(aminomethyl)-5-methylhexanoic acid.

Exemplary potassium channel openers include diazoxide, flupirtine, pinacidil, levcromakalim, rilimakalim, chromakalim, PCO-400 and SKP-450 (2-[2''(1'', 3''-dioxolone)-2-methyl]-4-(2'-oxo-1'-pyrrolidinyl)-6-nitro-2H-1-benzopyran).

Exemplary AMPA/kainate receptor antagonists include 6-cyano-7-nitroquinoxalin-2,3-di-one (CNQX); 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX); 6,7-dinitroquinoxaline-2,3-dione (DNQX); 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride; and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-[f]quinoxaline.

Exemplary sodium channel antagonists include ajmaline, procainamide, flecainide and riluzole.

Exemplary matrix-metalloprotease inhibitors include 4-[4-(4-fluorophenoxy)benzenesulfon-ylamino]tetrahydropyran-4-carboxylic acid hydroxyamide; 5-Methyl-5-(4-(4'-fluorophenoxy)-phenoxy)-pyrimidine-2,4,6-trione; 5-n-Butyl-5-(4-(4'-fluorophenoxy)-phenoxy)-pyrimidine-2,4,6-trione and prinomistat.

Poly(ADP ribose) polymerase (PARP) is an abundant nuclear enzyme which is activated by DNA strand single breaks to synthesize poly (ADP ribose) from NAD. Under normal conditions, PARP is involved in base excision repair caused by oxidative stress via the activation and recruitment of DNA repair enzymes in the nucleus. Thus, PARP plays a role in cell necrosis and DNA repair. PARP also participates in regulating cytokine expression that mediates inflammation. Under conditions where DNA damage is excessive (such as by acute excessive exposure to a pathological insult), PARP is over-activated, resulting in cell-based energetic failure characterized by NAD depletion and leading to ATP consumption, cellular necrosis, tissue injury, and organ damage/failure. PARP is thought to contribute to neurodegeneration by depleting nicotinamide adenine dinucleotide (NAD⁺) which then reduces adenosine triphosphate (ATP; Cosi and Marien, Ann. N.Y. Acad. Sci., 890:227, 1999) contributing to cell death which can be prevented by PARP inhibitors. Exemplory PARP inhibitors can be found in Southan and Szabo, Current Medicinal Chemistry, 10:321, 2003.

Exemplary inhibitors of p38 MAP kinase and c-jun-N-terminal kinases include pyridyl imidazoles, such as PD 169316, isomeric PD 169316, SB 203580, SB 202190, SB 220026, and RWJ 67657. Others are described in US Patent 6,288,089, and incorporated by reference herein.

In an exemplary embodiment, a combination therapy for treating or preventing MS comprises a therapeutically effective amount of one or more sirtuin variant therapeutic agents and one or more of Avonex[®] (interferon beta-1a), Tysabri[®] (natalizumab), or Fumaderm[®] (BG-12/Oral Fumarate).

5 In another embodiment, a combination therapy for treating or preventing diabetic neuropathy or conditions associated therewith comprises a therapeutically effective amount of one or more sirtuin variant therapeutic agents and one or more of tricyclic antidepressants (TCAs) (including, for example, imipramine, amitriptyline, desipramine and nortriptyline), serotonin reuptake inhibitors (SSRIs)
10 (including, for example, fluoxetine, paroxetine, sertralene, and citalopram) and antiepileptic drugs (AEDs) (including, for example, gabapentin, carbamazepine, and topiramate).

In another embodiment, the invention provides a method for treating or preventing a polyglutamine disease using a combination comprising at least one
15 sirtuin variant therapeutic agent, e.g., a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide, and at least one HDAC I/II inhibitor. Examples of HDAC I/II inhibitors include hydroxamic acids, cyclic peptides, benzamides, short-chain fatty acids, and depudecin.

Examples of hydroxamic acids and hydroxamic acid derivatives, but are not
20 limited to, trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), oxamflatin, suberic bishydroxamic acid (SBHA), m-carboxy-cinnamic acid bishydroxamic acid (CBHA), valproic acid and pyroxamide. TSA was isolated as an antifungal antibiotic (Tsuji et al (1976) J. Antibiot (Tokyo) 29:1-6) and found to be a potent inhibitor of mammalian HDAC (Yoshida et al. (1990) J. Biol. Chem.
25 265:17174-17179). The finding that TSA-resistant cell lines have an altered HDAC evidences that this enzyme is an important target for TSA. Other hydroxamic acid-based HDAC inhibitors, SAHA, SBHA, and CBHA are synthetic compounds that are able to inhibit HDAC at micromolar concentration or lower *in vitro* or *in vivo*. Glick et al. (1999) Cancer Res. 59:4392-4399. These hydroxamic acid-based HDAC
30 inhibitors all possess an essential structural feature: a polar hydroxamic terminal linked through a hydrophobic methylene spacer (e.g. 6 carbon at length) to another polar site which is attached to a terminal hydrophobic moiety (e.g., benzene ring).

Compounds developed having such essential features also fall within the scope of the hydroxamic acids that may be used as HDAC inhibitors.

Cyclic peptides used as HDAC inhibitors are mainly cyclic tetrapeptides. Examples of cyclic peptides include, but are not limited to, trapoxin A, apicidin and
5 depsipeptide. Trapoxin A is a cyclic tetrapeptide that contains a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety. Kijima et al. (1993) J. Biol. Chem. 268:22429-22435. Apicidin is a fungal metabolite that exhibits potent, broad-spectrum antiprotozoal activity and inhibits HDAC activity at nanomolar concentrations. Darkin-Rattray et al. (1996) Proc. Natl. Acad. Sci. USA. 93:13143-13147.
10 Depsipeptide is isolated from *Chromobacterium violaceum*, and has been shown to inhibit HDAC activity at micromolar concentrations.

Examples of benzamides include but are not limited to MS-27-275. Saito et al. (1990) Proc. Natl. Acad. Sci. USA. 96:4592-4597. Examples of short-chain fatty acids include but are not limited to butyrates (e.g., butyric acid, arginine butyrate
15 and phenylbutyrate (PB)). Newmark et al. (1994) Cancer Lett. 78:1-5; and Carducci et al. (1997) Anticancer Res. 17:3972-3973. In addition, depudecin which has been shown to inhibit HDAC at micromolar concentrations (Kwon et al. (1998) Proc. Natl. Acad. Sci. USA. 95:3356-3361) also falls within the scope of histone deacetylase inhibitor as described herein.

20 ***Blood Coagulation Disorders***

In other aspects, sirtuin variant therapeutic agents can be used to treat or prevent blood coagulation disorders (or hemostatic disorders). As used interchangeably herein, the terms “hemostasis”, “blood coagulation,” and “blood clotting” refer to the control of bleeding, including the physiological properties of
25 vasoconstriction and coagulation. Blood coagulation assists in maintaining the integrity of mammalian circulation after injury, inflammation, disease, congenital defect, dysfunction or other disruption. After initiation of clotting, blood coagulation proceeds through the sequential activation of certain plasma proenzymes to their enzyme forms (see, for example, Coleman, R. W. et al. (eds.) *Hemostasis and Thrombosis, Second Edition*, (1987)). These plasma glycoproteins, including
30 Factor XII, Factor XI, Factor IX, Factor X, Factor VII, and prothrombin, are zymogens of serine proteases. Most of these blood clotting enzymes are effective on

a physiological scale only when assembled in complexes on membrane surfaces with protein cofactors such as Factor VIII and Factor V. Other blood factors modulate and localize clot formation, or dissolve blood clots. Activated protein C is a specific enzyme that inactivates procoagulant components. Calcium ions are involved in many of the component reactions. Blood coagulation follows either the intrinsic pathway, where all of the protein components are present in blood, or the extrinsic pathway, where the cell-membrane protein tissue factor plays a critical role. Clot formation occurs when fibrinogen is cleaved by thrombin to form fibrin. Blood clots are composed of activated platelets and fibrin.

Further, the formation of blood clots does not only limit bleeding in the case of an injury (hemostasis), but may lead to serious organ damage and death in the context of atherosclerotic diseases by occlusion of an important artery or vein. Thrombosis is thus blood clot formation at the wrong time and place. It involves a cascade of complicated and regulated biochemical reactions between circulating blood proteins (coagulation factors), blood cells (in particular platelets), and elements of an injured vessel wall.

Accordingly, the present invention provides anticoagulation and antithrombotic treatments aiming at inhibiting the formation of blood clots in order to prevent or treat blood coagulation disorders, such as myocardial infarction, stroke, loss of a limb by peripheral artery disease or pulmonary embolism.

As used interchangeably herein, “modulating or modulation of hemostasis” and “regulating or regulation of hemostasis” includes the induction (e.g., stimulation or increase) of hemostasis, as well as the inhibition (e.g., reduction or decrease) of hemostasis.

In one aspect, the invention provides a method for reducing or inhibiting hemostasis in a subject by administering a sirtuin variant therapeutic agent. The compositions and methods disclosed herein are useful for the treatment or prevention of thrombotic disorders. As used herein, the term “thrombotic disorder” includes any disorder or condition characterized by excessive or unwanted coagulation or hemostatic activity, or a hypercoagulable state. Thrombotic disorders include diseases or disorders involving platelet adhesion and thrombus formation, and may manifest as an increased propensity to form thromboses, e.g., an increased

number of thromboses, thrombosis at an early age, a familial tendency towards thrombosis, and thrombosis at unusual sites. Examples of thrombotic disorders include, but are not limited to, thromboembolism, deep vein thrombosis, pulmonary embolism, stroke, myocardial infarction, miscarriage, thrombophilia associated with
5 anti-thrombin III deficiency, protein C deficiency, protein S deficiency, resistance to activated protein C, dysfibrinogenemia, fibrinolytic disorders, homocystinuria, pregnancy, inflammatory disorders, myeloproliferative disorders, arteriosclerosis, angina, e.g., unstable angina, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, cancer metastasis, sickle cell disease, glomerular
10 nephritis, and drug induced thrombocytopenia (including, for example, heparin induced thrombocytopenia). In addition, sirtuin variant therapeutic agents may be administered to prevent thrombotic events or to prevent re-occlusion during or after therapeutic clot lysis or procedures such as angioplasty or surgery.

In another embodiment, a combination drug regimen may include drugs or
15 compounds for the treatment or prevention of blood coagulation disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin variant therapeutic agents, e.g., one or more sirtuin variant polypeptides, one or more nucleic acids encoding sirtuin variant polypeptides, or combinations thereof, and one or more anti-coagulation or anti-
20 thrombosis agents. For example, one or more sirtuin variant therapeutic agents can be combined with an effective amount of one or more of: aspirin, heparin, and oral Warfarin that inhibits Vit K-dependent factors, low molecular weight heparins that inhibit factors X and II, thrombin inhibitors, inhibitors of platelet GP IIb/IIIa receptors, inhibitors of tissue factor (TF), inhibitors of human von Willebrand factor,
25 inhibitors of one or more factors involved in hemostasis (in particular in the coagulation cascade). In addition, sirtuin variant therapeutic agents can be combined with thrombolytic agents, such as t-PA, streptokinase, reptilase, TNK-t-PA, and staphylokinase.

Weight Control

30 In another aspect, sirtuin variant therapeutic agents may be used for treating or preventing weight gain or obesity in a subject. For example, sirtuin variant therapeutic agents may be used, for example, to treat or prevent hereditary obesity,

dietary obesity, hormone related obesity, obesity related to the administration of medication, to reduce the weight of a subject, or to reduce or prevent weight gain in a subject. A subject in need of such a treatment may be a subject who is obese, likely to become obese, overweight, or likely to become overweight. Subjects who
5 are likely to become obese or overweight can be identified, for example, based on family history, genetics, diet, activity level, medication intake, or various combinations thereof.

In yet other embodiments, sirtuin variant therapeutic agents may be administered to subjects suffering from a variety of other diseases and conditions
10 that may be treated or prevented by promoting weight loss in the subject. Such diseases include, for example, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, type 2 diabetes, insulin resistance, glucose intolerance, hyperinsulinemia, coronary heart disease, angina pectoris, congestive heart failure, stroke, gallstones, cholecystitis and cholelithiasis, gout, osteoarthritis, obstructive
15 sleep apnea and respiratory problems, some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation), bladder control problems (such as stress incontinence); uric acid nephrolithiasis; psychological disorders (such as depression, eating disorders, distorted body image, and low self esteem). Stunkard AJ, Wadden TA. (Editors) Obesity: theory and
20 therapy, Second Edition. New York: Raven Press, 1993. Finally, patients with AIDS can develop lipodystrophy or insulin resistance in response to combination therapies for AIDS.

In another embodiment, sirtuin variant therapeutic agents may be used for
25 inhibiting adipogenesis or fat cell differentiation, whether *in vitro* or *in vivo*. In particular, high circulating levels of insulin and/or insulin like growth factor (IGF) 1 will be prevented from recruiting preadipocytes to differentiate into adipocytes. Such methods may be used for treating or preventing obesity.

In other embodiments, sirtuin variant therapeutic agents may be used for
30 reducing appetite and/or increasing satiety, thereby causing weight loss or avoidance of weight gain. A subject in need of such a treatment may be a subject who is overweight, obese or a subject likely to become overweight or obese. The method

may comprise administering daily or, every other day, or once a week, a dose, e.g., in the form of a pill, to a subject. The dose may be an "appetite reducing dose."

A method for modulating weight may further comprise monitoring the weight of the subject and/or the level of sirtuin activity, for example, in adipose
5 tissue.

In an exemplary embodiment, sirtuin variant therapeutic agents may be administered as a combination therapy for treating or preventing weight gain or obesity. For example, one or more sirtuin variant therapeutic agents may be administered in combination with one or more anti-obesity agents. Exemplary anti-
10 obesity agents include, for example, phenylpropanolamine, ephedrine, pseudoephedrine, phentermine, a cholecystokinin-A agonist, a monoamine reuptake inhibitor (such as sibutramine), a sympathomimetic agent, a serotonergic agent (such as dexfenfluramine or fenfluramine), a dopamine agonist (such as bromocriptine), a melanocyte-stimulating hormone receptor agonist or mimetic, a melanocyte-
15 stimulating hormone analog, a cannabinoid receptor antagonist, a melanin concentrating hormone antagonist, the OB protein (leptin), a leptin analog, a leptin receptor agonist, a galanin antagonist or a GI lipase inhibitor or decriaser (such as orlistat). Other anorectic agents include bombesin agonists, dehydroepiandrosterone or analogs thereof, glucocorticoid receptor agonists and antagonists, orexin receptor
20 antagonists, urocortin binding protein antagonists, agonists of the glucagon-like peptide-1 receptor such as Exendin and ciliary neurotrophic factors such as Axokine.

In another embodiment, sirtuin variant therapeutic agents may be administered to reduce drug-induced weight gain. For example, sirtuin variant therapeutic agents may be administered as a combination therapy with medications
25 that may stimulate appetite or cause weight gain, in particular, weight gain due to factors other than water retention. Examples of medications that may cause weight gain, include for example, diabetes treatments, including, for example, sulfonylureas (such as glipizide and glyburide), thiazolidinediones (such as pioglitazone and rosiglitazone), meglitinides, nateglinide, repaglinide, sulphonylurea medicines, and
30 insulin; anti-depressants, including, for example, tricyclic antidepressants (such as amitriptyline and imipramine), irreversible monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), bupropion, paroxetine, and

mirtazapine; steroids, such as, for example, prednisone; hormone therapy; lithium carbonate; valproic acid; carbamazepine; chlorpromazine; thiothixene; beta blockers (such as propranolol); alpha blockers (such as clonidine, prazosin and terazosin); and contraceptives including oral contraceptives (birth control pills) or other
5 contraceptives containing estrogen and/or progesterone (Depo-Provera, Norplant, Ortho), testosterone or Megestrol. In another exemplary embodiment, sirtuin variant therapeutic agents may be administered as part of a smoking cessation program to prevent weight gain or reduce weight already gained.

Metabolic Disorders/Diabetes

10 In another aspect, sirtuin variant therapeutic agents may be used for treating or preventing a metabolic disorder, such as insulin-resistance, a pre-diabetic state, type II diabetes, and/or complications thereof. Administration of a sirtuin variant therapeutic agent may increase insulin sensitivity and/or decrease insulin levels in a subject. A subject in need of such a treatment may be a subject who has insulin
15 resistance or other precursor symptom of type II diabetes, who has type II diabetes, or who is likely to develop any of these conditions. For example, the subject may be a subject having insulin resistance, e.g., having high circulating levels of insulin and/or associated conditions, such as hyperlipidemia, dyslipogenesis, hypercholesterolemia, impaired glucose tolerance, high blood glucose sugar level,
20 other manifestations of syndrome X, hypertension, atherosclerosis and lipodystrophy.

In an exemplary embodiment, sirtuin variant therapeutic agents may be administered as a combination therapy for treating or preventing a metabolic disorder. For example, one or more sirtuin variant therapeutic agents may be
25 administered in combination with one or more anti-diabetic agents. Exemplary anti-diabetic agents include, for example, an aldose reductase inhibitor, a glycogen phosphorylase inhibitor, a sorbitol dehydrogenase inhibitor, a protein tyrosine phosphatase 1B inhibitor, a dipeptidyl protease inhibitor, insulin (including orally bioavailable insulin preparations), an insulin mimetic, metformin, acarbose, a
30 peroxisome proliferator-activated receptor- γ (PPAR- γ) ligand such as troglitazone, rosiglitazone, pioglitazone or GW-1929, a sulfonylurea, glipazide, glyburide, or chlorpropamide wherein the amounts of the first and second compounds result in a

therapeutic effect. Other anti-diabetic agents include a glucosidase inhibitor, a glucagon-like peptide-1 (GLP-1), insulin, a PPAR α/γ dual agonist, a meglitimide and an $\alpha P2$ inhibitor. In an exemplary embodiment, an anti-diabetic agent may be a dipeptidyl peptidase IV (DP-IV or DPP-IV) inhibitor, such as, for example LAF237
5 from Novartis (NVP DPP728; 1-[[[2-[(5-cyanopyridin-2-yl)amino] ethyl]amino]acetyl]-2- cyano-(S)- pyrrolidine) or MK-04301 from Merck (see e.g., Hughes et al., Biochemistry 38: 11597-603 (1999)).

Inflammatory Diseases

In other aspects, sirtuin variant therapeutic agents can be used to treat or
10 prevent a disease or disorder associated with inflammation. Sirtuin variant therapeutic agents may be administered prior to the onset of, at, or after the initiation of inflammation. When used prophylactically, the compounds are preferably provided in advance of any inflammatory response or symptom. Administration of the compounds may prevent or attenuate inflammatory responses
15 or symptoms.

Exemplary inflammatory conditions include, for example, multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, degenerative joint disease, spondouloarthropathies, gouty arthritis, systemic lupus erythematosus, juvenile arthritis, rheumatoid arthritis, osteoarthritis, osteoporosis, diabetes (e.g., insulin
20 dependent diabetes mellitus or juvenile onset diabetes), menstrual cramps, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, Crohn's disease, mucous colitis, ulcerative colitis, gastritis, esophagitis, pancreatitis, peritonitis, Alzheimer's disease, shock, ankylosing spondylitis, gastritis, conjunctivitis, pancreatis (acute or chronic), multiple organ injury syndrome (e.g., secondary to
25 septicemia or trauma), myocardial infarction, atherosclerosis, stroke, reperfusion injury (e.g., due to cardiopulmonary bypass or kidney dialysis), acute glomerulonephritis, vasculitis, thermal injury (i.e., sunburn), necrotizing enterocolitis, granulocyte transfusion associated syndrome, and/or Sjogren's syndrome. Exemplary inflammatory conditions of the skin include, for example,
30 eczema, atopic dermatitis, contact dermatitis, urticaria, schleroderma, psoriasis, and dermatosis with acute inflammatory components.

In another embodiment, sirtuin variant therapeutic agents may be used to treat or prevent allergies and respiratory conditions, including asthma, bronchitis, pulmonary fibrosis, allergic rhinitis, oxygen toxicity, emphysema, chronic bronchitis, acute respiratory distress syndrome, and any chronic obstructive pulmonary disease (COPD). The compounds may be used to treat chronic hepatitis infection, including hepatitis B and hepatitis C.

Additionally, sirtuin variant therapeutic agents may be used to treat autoimmune diseases and/or inflammation associated with autoimmune diseases such as organ-tissue autoimmune diseases (e.g., Raynaud's syndrome), scleroderma, myasthenia gravis, transplant rejection, endotoxin shock, sepsis, psoriasis, eczema, dermatitis, multiple sclerosis, autoimmune thyroiditis, uveitis, systemic lupus erythematosus, Addison's disease, autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), and Grave's disease.

In certain embodiments, one or more sirtuin variant therapeutic agents may be taken alone or in combination with other compounds useful for treating or preventing inflammation. Exemplary anti-inflammatory agents include, for example, steroids (e.g., cortisol, cortisone, fludrocortisone, prednisone, 6 α -methylprednisone, triamcinolone, betamethasone or dexamethasone), nonsteroidal antiinflammatory drugs (NSAIDS (e.g., aspirin, acetaminophen, tolmetin, ibuprofen, mefenamic acid, piroxicam, nabumetone, rofecoxib, celecoxib, etodolac or nimesulide). In another embodiment, the other therapeutic agent is an antibiotic (e.g., vancomycin, penicillin, amoxicillin, ampicillin, cefotaxime, ceftriaxone, cefixime, rifampinmetronidazole, doxycycline or streptomycin).

In another embodiment, the other therapeutic agent is a PDE4 inhibitor (e.g., roflumilast or rolipram). In another embodiment, the other therapeutic agent is an antihistamine (e.g., cyclizine, hydroxyzine, promethazine or diphenhydramine). In another embodiment, the other therapeutic agent is an anti-malarial (e.g., artemisinin, artemether, artsunate, chloroquine phosphate, mefloquine hydrochloride, doxycycline hyclate, proguanil hydrochloride, atovaquone or halofantrine). In one embodiment, the other therapeutic agent is drotrecogin alfa.

Further examples of anti-inflammatory agents include, for example, aceclofenac, acemetacin, e-acetamidocaproic acid, acetaminophen, acetaminosalol, acetanilide, acetylsalicylic acid, S-adenosylmethionine, alclofenac, alclometasone, alfentanil, algestone, allylprodine, alminoprofen, aloxiprin, alphaprodine, 5 aluminum bis(acetylsalicylate), amcinonide, amfenac, aminochlorthenoxazin, 3-amino-4-hydroxybutyric acid, 2-amino-4-picoline, aminopropylon, aminopyrine, amixetrine, ammonium salicylate, ampiroxicam, amtolmetin guacil, anileridine, antipyrine, antrafenine, apazone, beclomethasone, bendazac, benorylate, benoxaprofen, benzpiperylon, benzydamine, benzylmorphine, bermoprofen, 10 betamethasone, betamethasone-17-valerate, bezitramide, α -bisabolol, bromfenac, p-bromoacetanilide, 5-bromosalicylic acid acetate, bromosaligenin, bucetin, bucloxic acid, bucolome, budesonide, bufexamac, bumadizon, buprenorphine, butacetin, butibufen, butorphanol, carbamazepine, carbiphene, carprofen, carsalam, chlorobutanol, chloroprednisone, chlorthenoxazin, choline salicylate, cinchophen, 15 cinmetacin, ciramadol, clidanac, clobetasol, clocortolone, clometacin, clonitazene, clonixin, clopirac, cloprednol, clove, codeine, codeine methyl bromide, codeine phosphate, codeine sulfate, cortisone, cortivazol, cropropamide, crotethamide, cyclazocine, deflazacort, dehydrotestosterone, desomorphine, desonide, desoximetasone, dexamethasone, dexamethasone-21-isonicotinate, dexoxadrol, 20 dextromoramide, dextropropoxyphene, deoxycorticosterone, dezocine, diampromide, diamorphine, diclofenac, difenamizole, difenpiramide, diflorasone, diflucortolone, diflunisal, difluprednate, dihydrocodeine, dihydrocodeinone enol acetate, dihydromorphine, dihydroxyaluminum acetylsalicylate, dimenoxadol, dimepheptanol, dimethylthiambutene, dioxaphetyl butyrate, dipipanone, diprocetyl, 25 dipyrone, ditazol, droxicam, emorfazone, enfenamic acid, enoxolone, epirizole, eptazocine, etersalate, ethenzamide, ethoheptazine, ethoxazene, ethylmethylthiambutene, ethylmorphine, etodolac, etofenamate, etonitazene, eugenol, felbinac, fenbufen, fenclozic acid, fendosal, fenoprofen, fentanyl, fentiazac, fepradinol, feprazone, floctafenine, fluazacort, flucloronide, flufenamic acid, flumethasone, flunisolide, flunixin, flunoxaprofen, fluocinolone acetamide, 30 fluocinonide, fluocinolone acetamide, fluocortin butyl, fluocortolone, fluoresone,

fluorometholone, fluperolone, flupirtine, fluprednidene, fluprednisolone,
 fluproquazone, flurandrenolide, flurbiprofen, fluticasone, formocortal, fosfosal,
 gentisic acid, glafenine, glucametacin, glycol salicylate, guaiazulene, halcinonide,
 halobetasol, halometasone, haloprednone, heroin, hydrocodone, hydrocortamate,
 5 hydrocortisone, hydrocortisone acetate, hydrocortisone succinate, hydrocortisone
 hemisuccinate, hydrocortisone 21-lysinate, hydrocortisone cypionate,
 hydromorphone, hydroxypethidine, ibufenac, ibuprofen, ibuproxam, imidazole
 salicylate, indomethacin, indoprofen, isofezolac, isoflupredone, isoflupredone
 acetate, isoladol, isomethadone, isonixin, isoxepac, isoxicam, ketobemidone,
 10 ketoprofen, ketorolac, p-lactophenetide, lefetamine, levallorphan, levorphanol,
 levophenacyl-morphan, lofentanil, lonazolac, lornoxicam, loxoprofen, lysine
 acetylsalicylate, mazipredone, meclofenamic acid, medrysone, mefenamic acid,
 meloxicam, meperidine, meprednisone, meptazinol, mesalamine, metazocine,
 methadone, methotrimeprazine, methylprednisolone, methylprednisolone acetate,
 15 methylprednisolone sodium succinate, methylprednisolone suleptnate, metiazinic
 acid, metofoline, metopon, mofebutazone, mofezolac, mometasone, morazone,
 morphine, morphine hydrochloride, morphine sulfate, morpholine salicylate,
 myrophine, nabumetone, nalbuphine, nalorphine, 1-naphthyl salicylate, naproxen,
 narceine, nefopam, nicomorphine, nifenazone, niflumic acid, nimesulide, 5'-nitro-
 20 2'-propoxyacetanilide, norlevorphanol, normethadone, normorphine, norpipanone,
 olsalazine, opium, oxaceprol, oxametacine, oxaprozin, oxycodone, oxymorphone,
 oxyphenbutazone, papaveretum, paramethasone, paranyline, parsalimide,
 pentazocine, perisoxal, phenacetin, phenadoxone, phenazocine, phenazopyridine
 hydrochloride, phenocoll, phenoperidine, phenopyrazone, phenomorphan, phenyl
 25 acetylsalicylate, phenylbutazone, phenyl salicylate, phenyramidol, piketoprofen,
 piminodine, pipebuzone, piperylone, pirazolac, piritramide, piroxicam, pirprofen,
 pranoprofen, prednicarbate, prednisolone, prednisone, prednival, prednylidene,
 proglumetacin, proheptazine, promedol, propacetamol, properidine, propiram,
 propoxyphene, propyphenazone, proquazone, protizinic acid, proxazole,
 30 ramifenazone, remifentanil, rimazolium metilsulfate, salacetamide, salicin,
 salicylamide, salicylamide o-acetic acid, salicylic acid, salicylsulfuric acid,
 salsalate, salverine, simetride, sufentanil, sulfasalazine, sulindac, superoxide

dismutase, suprofen, suxibuzone, talniflumate, tenidap, tenoxicam, terofenamate, tetrandrine, thiazolinobutazone, tiaprofenic acid, tiaramide, tilidine, tinoridine, tixocortol, tolfenamic acid, tolmetin, tramadol, triamcinolone, triamcinolone acetonide, tropesin, viminol, xenbucin, ximoprofen, zaltoprofen and zomepirac.

5 In an exemplary embodiment, sirtuin variant therapeutic agents may be administered with a selective COX-2 inhibitor for treating or preventing inflammation. Exemplary selective COX-2 inhibitors include, for example, deracoxib, parecoxib, celecoxib, valdecoxib, rofecoxib, etoricoxib, lumiracoxib, 2-(3,5-difluorophenyl)-3-- [4-(methylsulfonyl)phenyl]-2-cyclopenten-1-one, (S)-6,8-
10 dichloro-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid, 2-(3,4-difluorophenyl)-4-(3-- hydroxy-3-methyl-1-butoxy)-5-[4-(methylsulfonyl)phenyl]-3-(2H)-pyridazinone, 4-[5-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide, tert-butyl 1 benzyl-4-[(4-oxopiperidin-1-yl)sulfonyl]piperidine-4-carboxylate, 4-[5-(phenyl)-3-(trifluoromethyl)-1H-
15 pyrazol-1-yl]benzenesulfonamide, salts and prodrugs thereof.

Flushing

In another aspect, sirtuin variant therapeutic agents may be used for reducing the incidence or severity of flushing and/or hot flashes which are symptoms of a disorder. For instance, the subject method includes the use of sirtuin variant
20 therapeutic agents, alone or in combination with other agents, for reducing incidence or severity of flushing and/or hot flashes in cancer patients. In other embodiments, the method provides for the use of sirtuin variant therapeutic agents to reduce the incidence or severity of flushing and/or hot flashes in menopausal and post-menopausal woman.

25 In another aspect, sirtuin variant therapeutic agents may be used as a therapy for reducing the incidence or severity of flushing and/or hot flashes which are side-effects of another drug therapy, e.g., drug-induced flushing. In certain embodiments, a method for treating and/or preventing drug-induced flushing comprises administering to a patient in need thereof a formulation comprising at
30 least one flushing inducing compound and at least one sirtuin variant therapeutic agent. In other embodiments, a method for treating drug induced flushing comprises separately administering one or more compounds that induce flushing and one or

more sirtuin variant therapeutic agents, e.g., wherein the sirtuin variant therapeutic agents and flushing inducing agent have not been formulated in the same compositions. When using separate formulations, the sirtuin variant therapeutic agents may be administered (1) at the same as administration of the flushing inducing agent, (2) intermittently with the flushing inducing agent, (3) staggered relative to administration of the flushing inducing agent, (4) prior to administration of the flushing inducing agent, (5) subsequent to administration of the flushing inducing agent, and (6) various combination thereof. Exemplary flushing inducing agents include, for example, niacin, faloixifene, antidepressants, anti-psychotics, chemotherapeutics, calcium channel blockers, and antibiotics.

In one embodiment, sirtuin variant therapeutic agents may be used to reduce flushing side effects of a vasodilator or an antilipemic agent (including anticholesteremic agents and lipotropic agents). In an exemplary embodiment, a sirtuin variant therapeutic agent may be used to reduce flushing associated with the administration of niacin.

Nicotinic acid, 3-pyridinecarboxylic acid or niacin, is an antilipidemic agent that is marketed under, for example, the trade names Nicolac[®], SloNiacin[®], Nicobid[®] and Time Release Niacin[®]. Nicotinic acid has been used for many years in the treatment of lipidemic disorders such as hyperlipidemia, hypercholesterolemia and atherosclerosis. This compound has long been known to exhibit the beneficial effects of reducing total cholesterol, low density lipoproteins or "LDL cholesterol," triglycerides and apolipoprotein a (Lp(a)) in the human body, while increasing desirable high density lipoproteins or "HDL cholesterol".

Typical doses range from about 1 gram to about 3 grams daily. Nicotinic acid is normally administered two to four times per day after meals, depending upon the dosage form selected. Nicotinic acid is currently commercially available in two dosage forms. One dosage form is an immediate or rapid release tablet which should be administered three or four times per day. Immediate release ("IR") nicotinic acid formulations generally release nearly all of their nicotinic acid within about 30 to 60 minutes following ingestion. The other dosage form is a sustained release form which is suitable for administration two to four times per day. In contrast to IR formulations, sustained release ("SR") nicotinic acid formulations are designed to

release significant quantities of drug for absorption into the blood stream over specific timed intervals in order to maintain therapeutic levels of nicotinic acid over an extended period such as 12 or 24 hours after ingestion.

As used herein, the term "nicotinic acid" is meant to encompass nicotinic
5 acid or a compound other than nicotinic acid itself which the body metabolizes into nicotinic acid, thus producing essentially the same effect as nicotinic acid. Exemplary compounds that produce an effect similar to that of nicotinic acid include, for example, nicotiny alcohol tartrate, d-glucitol hexanicotinate, aluminum nicotinate, niceritrol and d,1-alpha-tocopheryl nicotinate. Each such compound will
10 be collectively referred to herein as "nicotinic acid."

In another embodiment, the invention provides a method for treating and/or preventing hyperlipidemia with reduced flushing side effects. The method comprises the steps of administering to a subject in need thereof a therapeutically effective amount of nicotinic acid and a sirtuin variant therapeutic agent in an amount
15 sufficient to reduce flushing. In an exemplary embodiment, the nicotinic acid and/or sirtuin variant therapeutic agent may be administered nocturnally.

In another representative embodiment, the method involves the use of sirtuin variant therapeutic agents to reduce flushing side effects of raloxifene. Raloxifene acts like estrogen in certain places in the body, but is not a hormone. It helps prevent
20 osteoporosis in women who have reached menopause. Osteoporosis causes bones to gradually grow thin, fragile, and more likely to break. Evista slows down the loss of bone mass that occurs with menopause, lowering the risk of spine fractures due to osteoporosis. A common side effect of raloxifene is hot flashes (sweating and flushing). This can be uncomfortable for women who already have hot flashes due to
25 menopause.

In another representative embodiment, the method involves the use of sirtuin variant therapeutic agents to reduce flushing side effects of antidepressants or anti-
psychotic agent. For instance, sirtuin variant therapeutic agents can be used in conjunction (administered separately or together) with a serotonin reuptake
30 inhibitor, a 5HT2 receptor antagonist, an anticonvulsant, a norepinephrine reuptake inhibitor, an α -adrenoreceptor antagonist, an NK-3 antagonist, an NK-1 receptor

antagonist, a PDE4 inhibitor, an Neuropeptide Y5 Receptor Antagonists, a D4 receptor antagonist, a 5HT1A receptor antagonist, a 5HT1D receptor antagonist, a CRF antagonist, a monoamine oxidase inhibitor, or a sedative-hypnotic drug.

5 In certain embodiments, sirtuin variant therapeutic agents may be used as part of a treatment with a serotonin reuptake inhibitor (SRI) to reduce flushing. In certain preferred embodiments, the SRI is a selective serotonin reuptake inhibitor (SSRI), such as a fluoxetinoid (fluoxetine, norfluoxetine) or a nefazodonoid (nefazodone, hydroxynefazodone, oxonefazodone). Other exemplary SSRI's include duloxetine, venlafaxine, milnacipran, citalopram, fluvoxamine, paroxetine
10 and sertraline. The sirtuin variant therapeutic agent can also be used as part of a treatment with sedative-hypnotic drug, such as selected from the group consisting of a benzodiazepine (such as alprazolam, chlordiazepoxide, clonazepam, chlorazepate, clobazam, diazepam, halazepam, lorazepam, oxazepam and prazepam), zolpidem, and barbiturates. In still other embodiments, a sirtuin variant therapeutic agent may
15 be used as part of a treatment with a 5-HT1A receptor partial agonist, such as, for example, buspirone, flesinoxan, gepirone or ipsapirone. Sirtuin variant therapeutic agents can also be used as part of a treatment with a norepinephrine reuptake inhibitor, such as, for example, tertiary amine tricyclics and secondary amine tricyclics. Exemplary tertiary amine tricyclic include amitriptyline, clomipramine, doxepin,
20 imipramine and trimipramine. Exemplary secondary amine tricyclic include amoxapine, desipramine, maprotiline, nortriptyline and protriptyline. In certain embodiments, sirtuin variant therapeutic agents may be used as part of a treatment with a monoamine oxidase inhibitor, such as, for example, isocarboxazid, phenelzine, tranylcypromine, selegiline or moclobemide.

25 In still another representative embodiment, sirtuin variant therapeutic agents may be used to reduce flushing side effects of chemotherapeutic agents, such as cyclophosphamide, tamoxifen.

In another embodiment, sirtuin variant therapeutic agents may be used to reduce flushing side effects of calcium channel blockers, such as amlodipine.

30 In another embodiment, sirtuin variant therapeutic agents may be used to reduce flushing side effects of antibiotics. For example, sirtuin variant therapeutic agents can be used in combination with levofloxacin. Levofloxacin is used to treat

infections of the sinuses, skin, lungs, ears, airways, bones, and joints caused by susceptible bacteria. Levofloxacin also is frequently used to treat urinary infections, including those resistant to other antibiotics, as well as prostatitis. Levofloxacin is effective in treating infectious diarrheas caused by *E. coli*, campylobacter jejuni, and shigella bacteria. Levofloxacin also can be used to treat various obstetric infections, including mastitis.

Ocular Disorders

One aspect of the present invention is a method for inhibiting, reducing or otherwise treating vision impairment by administering to a patient a therapeutic dosage of a sirtuin variant therapeutic agent.

In certain aspects of the invention, the vision impairment is caused by damage to the optic nerve or central nervous system. In particular embodiments, optic nerve damage is caused by high intraocular pressure, such as that created by glaucoma. In other particular embodiments, optic nerve damage is caused by swelling of the nerve, which is often associated with an infection or an immune (e.g., autoimmune) response such as in optic neuritis.

Glaucoma describes a group of disorders which are associated with a visual field defect, cupping of the optic disc, and optic nerve damage. These are commonly referred to as glaucomatous optic neuropathies. Most glaucomas are usually, but not always, associated with a rise in intraocular pressure. Exemplary forms of glaucoma include Glaucoma and Penetrating Keratoplasty, Acute Angle Closure, Chronic Angle Closure, Chronic Open Angle, Angle Recession, Aphakic and Pseudophakic, Drug-Induced, Hyphema, Intraocular Tumors, Juvenile, Lens-Particle, Low Tension, Malignant, Neovascular, Phacolytic, Phacomorphic, Pigmentary, Plateau Iris, Primary Congenital, Primary Open Angle, Pseudoexfoliation, Secondary Congenital, Adult Suspect, Unilateral, Uveitic, Ocular Hypertension, Ocular Hypotony, Posner-Schlossman Syndrome and Scleral Expansion Procedure in Ocular Hypertension & Primary Open-angle Glaucoma.

Intraocular pressure can also be increased by various surgical procedures, such as phacoemulsification (i.e., cataract surgery) and implantation of structures such as an artificial lens. In addition, spinal surgeries in particular, or any surgery in

which the patient is prone for an extended period of time can lead to increased interocular pressure.

Optic neuritis (ON) is inflammation of the optic nerve and causes acute loss of vision. It is highly associated with multiple sclerosis (MS) as 15-25% of MS patients initially present with ON, and 50-75% of ON patients are diagnosed with MS. ON is also associated with infection (e.g., viral infection, meningitis, syphilis), inflammation (e.g., from a vaccine), infiltration and ischemia.

Another condition leading to optic nerve damage is anterior ischemic optic neuropathy (AION). There are two types of AION. Arteritic AION is due to giant cell arteritis (vasculitis) and leads to acute vision loss. Non-arteritic AION encompasses all cases of ischemic optic neuropathy other than those due to giant cell arteritis. The pathophysiology of AION is unclear although it appears to incorporate both inflammatory and ischemic mechanisms.

Other damage to the optic nerve is typically associated with demyelination, inflammation, ischemia, toxins, or trauma to the optic nerve. Exemplary conditions where the optic nerve is damaged include Demyelinating Optic Neuropathy (Optic Neuritis, Retrobulbar Optic Neuritis), Optic Nerve Sheath Meningioma, Adult Optic Neuritis, Childhood Optic Neuritis, Anterior Ischemic Optic Neuropathy, Posterior Ischemic Optic Neuropathy, Compressive Optic Neuropathy, Papilledema, Pseudopapilledema and Toxic/Nutritional Optic Neuropathy.

Other neurological conditions associated with vision loss, albeit not directly associated with damage to the optic nerve, include Amblyopia, Bells Palsy, Chronic Progressive External Ophthalmoplegia, Multiple Sclerosis, Pseudotumor Cerebri and Trigeminal Neuralgia.

In certain aspects of the invention, the vision impairment is caused by retinal damage. In particular embodiments, retinal damage is caused by disturbances in blood flow to the eye (e.g., arteriosclerosis, vasculitis). In particular embodiments, retinal damage is caused by disruption of the macula (e.g., exudative or non-exudative macular degeneration).

Exemplary retinal diseases include Exudative Age Related Macular Degeneration, Nonexudative Age Related Macular Degeneration, Retinal Electronic Prosthesis and RPE Transplantation Age Related Macular Degeneration, Acute

Multifocal Placoid Pigment Epitheliopathy, Acute Retinal Necrosis, Best Disease, Branch Retinal Artery Occlusion, Branch Retinal Vein Occlusion, Cancer Associated and Related Autoimmune Retinopathies, Central Retinal Artery Occlusion, Central Retinal Vein Occlusion, Central Serous Chorioretinopathy, Eales Disease, Epimacular Membrane, Lattice Degeneration, Macroaneurysm, Diabetic Macular Edema, Irvine-Gass Macular Edema, Macular Hole, Subretinal Neovascular Membranes, Diffuse Unilateral Subacute Neuroretinitis, Nonpseudophakic Cystoid Macular Edema, Presumed Ocular Histoplasmosis Syndrome, Exudative Retinal Detachment, Postoperative Retinal Detachment, Proliferative Retinal Detachment, Rhegmatogenous Retinal Detachment, Tractional Retinal Detachment, Retinitis Pigmentosa, CMV Retinitis, Retinoblastoma, Retinopathy of Prematurity, Birdshot Retinopathy, Background Diabetic Retinopathy, Proliferative Diabetic Retinopathy, Hemoglobinopathies Retinopathy, Purtscher Retinopathy, Valsalva Retinopathy, Juvenile Retinoschisis, Senile Retinoschisis, Terson Syndrome and White Dot Syndromes.

Other exemplary diseases include ocular bacterial infections (e.g. conjunctivitis, keratitis, tuberculosis, syphilis, gonorrhea), viral infections (e.g. Ocular Herpes Simplex Virus, Varicella Zoster Virus, Cytomegalovirus retinitis, Human Immunodeficiency Virus (HIV)) as well as progressive outer retinal necrosis secondary to HIV or other HIV-associated and other immunodeficiency-associated ocular diseases. In addition, ocular diseases include fungal infections (e.g. Candida choroiditis, histoplasmosis), protozoal infections (e.g. toxoplasmosis) and others such as ocular toxocariasis and sarcoidosis.

One aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing treatment with a chemotherapeutic drug (e.g., a neurotoxic drug, a drug that raises intraocular pressure such as a steroid), by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin variant therapeutic agent.

Another aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing surgery, including ocular or other surgeries performed in the prone position such as spinal cord surgery, by administering to the subject in need of such treatment a therapeutic dosage of a

sirtuin variant therapeutic agent. Ocular surgeries include cataract, iridotomy and lens replacements.

Another aspect of the invention is the treatment, including inhibition and prophylactic treatment, of age related ocular diseases include cataracts, dry eye, 5 retinal damage and the like, by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin variant therapeutic agent.

The formation of cataracts is associated with several biochemical changes in the lens of the eye, such as decreased levels of antioxidants ascorbic acid and glutathione, increased lipid, amino acid and protein oxidation, increased sodium and 10 calcium, loss of amino acids and decreased lens metabolism. The lens, which lacks blood vessels, is suspended in extracellular fluids in the anterior part of the eye. Nutrients, such as ascorbic acid, glutathione, vitamin E, selenium, bioflavonoids and carotenoids are required to maintain the transparency of the lens. Low levels of selenium results in an increase of free radical-inducing hydrogen peroxide, which is 15 neutralized by the selenium-dependent antioxidant enzyme glutathione peroxidase. Lens-protective glutathione peroxidase is also dependent on the amino acids methionine, cysteine, glycine and glutamic acid.

Cataracts can also develop due to an inability to properly metabolize galactose found in dairy products that contain lactose, a disaccharide composed of 20 the monosaccharide galactose and glucose. Cataracts can be prevented, delayed, slowed and possibly even reversed if detected early and metabolically corrected.

Retinal damage is attributed, *inter alia*, to free radical initiated reactions in glaucoma, diabetic retinopathy and age-related macular degeneration (AMD). The eye is a part of the central nervous system and has limited regenerative capability. 25 The retina is composed of numerous nerve cells which contain the highest concentration of polyunsaturated fatty acids (PFA) and subject to oxidation. Free radicals are generated by UV light entering the eye and mitochondria in the rods and cones, which generate the energy necessary to transform light into visual impulses. Free radicals cause peroxidation of the PFA by hydroxyl or superoxide radicals 30 which in turn propagate additional free radicals. The free radicals cause temporary or permanent damage to retinal tissue.

Glaucoma is usually viewed as a disorder that causes an elevated intraocular pressure (IOP) that results in permanent damage to the retinal nerve fibers, but a sixth of all glaucoma cases do not develop an elevated IOP. This disorder is now perceived as one of reduced vascular perfusion and an increase in neurotoxic factors.

5 Recent studies have implicated elevated levels of glutamate, nitric oxide and peroxynitrite in the eye as the causes of the death of retinal ganglion cells. Neuroprotective agents may be the future of glaucoma care. For example, nitric oxide synthase inhibitors block the formation of peroxynitrite from nitric oxide and superoxide. In a recent study, animals treated with aminoguanidine, a nitric oxide
10 synthase inhibitor, had a reduction in the loss of retinal ganglion cells. It was concluded that nitric oxide in the eye caused cytotoxicity in many tissues and neurotoxicity in the central nervous system.

Diabetic retinopathy occurs when the underlying blood vessels develop microvascular abnormalities consisting primarily of microaneurysms and intraretinal
15 hemorrhages. Oxidative metabolites are directly involved with the pathogenesis of diabetic retinopathy and free radicals augment the generation of growth factors that lead to enhanced proliferative activity. Nitric oxide produced by endothelial cells of the vessels may also cause smooth muscle cells to relax and result in vasodilation of segments of the vessel. Ischemia and hypoxia of the retina occur after thickening of
20 the arterial basement membrane, endothelial proliferation and loss of pericytes. The inadequate oxygenation causes capillary obliteration or nonperfusion, arteriolar-venular shunts, sluggish blood flow and an impaired ability of RBCs to release oxygen. Lipid peroxidation of the retinal tissues also occurs as a result of free radical damage.

25 The macula is responsible for our acute central vision and composed of light-sensing cells (cones) while the underlying retinal pigment epithelium (RPE) and choroid nourish and help remove waste materials. The RPE nourishes the cones with the vitamin A substrate for the photosensitive pigments and digests the cones shed outer tips. RPE is exposed to high levels of UV radiation, and secretes factors that
30 inhibit angiogenesis. The choroid contains a dense vascular network that provides nutrients and removes the waste materials.

In AMD, the shed cone tips become indigestible by the RPE, where the cells swell and die after collecting too much undigested material. Collections of undigested waste material, called drusen, form under the RPE. Phototoxic damage also causes the accumulation of lipofuscin in RPE cells. The intracellular lipofuscin and accumulation of drusen in Bruch's membrane interferes with the transport of oxygen and nutrients to the retinal tissues, and ultimately leads to RPE and photoreceptor dysfunction. In exudative AMD, blood vessels grow from the choriocapillaris through defects in Bruch's membrane and may grow under the RPE, detaching it from the choroid, and leaking fluid or bleeding.

Macular pigment, one of the protective factors that prevent sunlight from damaging the retina, is formed by the accumulation of nutritionally derived carotenoids, such as lutein, the fatty yellow pigment that serves as a delivery vehicle for other important nutrients and zeaxanthin. Antioxidants such as vitamins C and E, beta-carotene and lutein, as well as zinc, selenium and copper, are all found in the healthy macula. In addition to providing nourishment, these antioxidants protect against free radical damage that initiates macular degeneration.

Another aspect of the invention is the prevention or treatment of damage to the eye caused by stress, chemical insult or radiation, by administering to the subject in need of such treatment a therapeutic dosage of a sirtuin variant therapeutic agent. Radiation or electromagnetic damage to the eye can include that caused by CRT's or exposure to sunlight or UV.

In one embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of ocular disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin variant therapeutic agents and one or more therapeutic agents for the treatment of an ocular disorder. For example, one or more sirtuin variant therapeutic agents can be combined with an effective amount of one or more of: an agent that reduces intraocular pressure, an agent for treating glaucoma, an agent for treating optic neuritis, an agent for treating CMV Retinopathy, an agent for treating multiple sclerosis, and/or an antibiotic, etc.

In one embodiment, a sirtuin variant therapeutic agent can be administered in conjunction with a therapy for reducing intraocular pressure. One group of therapies

involves blocking aqueous production. For example, topical beta-adrenergic antagonists (timolol and betaxolol) decrease aqueous production. Topical timolol causes IOP to fall in 30 minutes with peak effects in 1-2 hours. A reasonable regimen is Timoptic 0.5%, one drop every 30 minutes for 2 doses. The carbonic anhydrase inhibitor, acetazolamide, also decreases aqueous production and should be given in conjunction with topical beta-antagonists. An initial dose of 500 mg is administered followed by 250 mg every 6 hours. This medication may be given orally, intramuscularly, or intravenously. In addition, alpha 2-agonists (e.g., Apraclonidine) act by decreasing aqueous production. Their effects are additive to topically administered beta-blockers. They have been approved for use in controlling an acute rise in pressure following anterior chamber laser procedures, but has been reported effective in treating acute closed-angle glaucoma. A reasonable regimen is 1 drop every 30 minutes for 2 doses.

A second group of therapies for reducing intraocular pressure involve reducing vitreous volume. Hyperosmotic agents can be used to treat an acute attack. These agents draw water out of the globe by making the blood hyperosmolar. Oral glycerol in a dose of 1 mL/kg in a cold 50% solution (mixed with lemon juice to make it more palatable) often is used. Glycerol is converted to glucose in the liver; persons with diabetes may need additional insulin if they become hyperglycemic after receiving glycerol. Oral isosorbide is a metabolically inert alcohol that also can be used as an osmotic agent for patients with acute angle-closure glaucoma. Usual dose is 100 g taken p.o. (220 cc of a 45% solution). This inert alcohol should not be confused with isosorbide dinitrate, a nitrate-based cardiac medication used for angina and for congestive heart failure. Intravenous mannitol in a dose of 1.0-1.5 mg/kg also is effective and is well tolerated in patients with nausea and vomiting. These hyperosmotic agents should be used with caution in any patient with a history of congestive heart failure.

A third group of therapies involve facilitating aqueous outflow from the eye. Miotic agents pull the iris from the iridocorneal angle and may help to relieve the obstruction of the trabecular meshwork by the peripheral iris. Pilocarpine 2% (blue eyes)-4% (brown eyes) can be administered every 15 minutes for the first 1-2 hours.

More frequent administration or higher doses may precipitate a systemic cholinergic crisis. NSAIDS are sometimes used to reduce inflammation.

Exemplary therapeutic agents for reducing intraocular pressure include ALPHAGAN® P (Allergan) (brimonidine tartrate ophthalmic solution), AZOPT®
5 (Alcon) (brinzolamide ophthalmic suspension), BETAGAN® (Allergan) (levobunolol hydrochloride ophthalmic solution, USP), BETIMOL® (Vistakon) (timolol ophthalmic solution), BETOPTIC S® (Alcon) (betaxolol HCl), BRIMONIDINE TARTRATE (Bausch & Lomb), CARTEOLOL HYDROCHLORIDE (Bausch & Lomb), COSOPT® (Merck) (dorzolamide
10 hydrochloride-timolol maleate ophthalmic solution), LUMIGAN® (Allergan) (bimatoprost ophthalmic solution), OPTIPRANOLOL® (Bausch & Lomb) (metipranolol ophthalmic solution), TIMOLOL GFS (Falcon) (timolol maleate ophthalmic gel forming solution), TIMOPTIC® (Merck) (timolol maleate ophthalmic solution), TRAVATAN® (Alcon) (travoprost ophthalmic solution),
15 TRUSOPT® (Merck) (dorzolamide hydrochloride ophthalmic solution) and XALATAN® (Pharmacia & Upjohn) (latanoprost ophthalmic solution).

In one embodiment, a sirtuin variant therapeutic agent can be administered in conjunction with a therapy for treating and/or preventing glaucoma. An example of a glaucoma drug is DARANIDE® Tablets (Merck) (Dichlorphenamide).

20 In one embodiment, a sirtuin variant therapeutic agent can be administered in conjunction with a therapy for treating and/or preventing optic neuritis. Examples of drugs for optic neuritis include DECADRON® Phosphate Injection (Merck) (Dexamethasone Sodium Phosphate), DEPO-MEDROL® (Pharmacia & Upjohn)(methylprednisolone acetate), HYDROCORTONE® Tablets (Merck)
25 (Hydrocortisone), ORAPRED® (Biomarin) (prednisolone sodium phosphate oral solution) and PEDIAPRED® (Celltech) (prednisolone sodium phosphate, USP).

In one embodiment, a sirtuin variant therapeutic agent can be administered in conjunction with a therapy for treating and/or preventing CMV Retinopathy. Treatments for CMV retinopathy include CYTOVENE® (ganciclovir capsules) and
30 VALCYTE® (Roche Laboratories) (valganciclovir hydrochloride tablets).

In one embodiment, a sirtuin variant therapeutic agent can be administered in conjunction with a therapy for treating and/or preventing multiple sclerosis.

Examples of such drugs include DANTRIUM® (Procter & Gamble Pharmaceuticals) (dantrolene sodium), NOVANTRONE® (Serono) (mitoxantrone), AVONEX® (Biogen Idec) (Interferon beta-1a), BETASERON® (Berlex) (Interferon beta-1b), COPAXONE® (Teva Neuroscience) (glatiramer acetate injection) and REBIF® (Pfizer) (interferon beta-1a).

In addition, macrolide and/or mycophenolic acid, which has multiple activities, can be co-administered with a sirtuin variant therapeutic agent. Macrolide antibiotics include tacrolimus, cyclosporine, sirolimus, everolimus, ascomycin, erythromycin, azithromycin, clarithromycin, clindamycin, lincomycin, dirithromycin, josamycin, spiramycin, diacetyl-midecamycin, tylosin, roxithromycin, ABT-773, telithromycin, leucomycins, and lincosamide.

Mitochondrial-Associated Diseases and Disorders

In certain embodiments, the invention provides methods for treating diseases or disorders that would benefit from increased mitochondrial activity. The methods involve administering to a subject in need thereof a therapeutically effective amount of a sirtuin variant therapeutic agent. Increased mitochondrial activity refers to increasing activity of the mitochondria while maintaining the overall numbers of mitochondria (e.g., mitochondrial mass), increasing the numbers of mitochondria thereby increasing mitochondrial activity (e.g., by stimulating mitochondrial biogenesis), or combinations thereof. In certain embodiments, diseases and disorders that would benefit from increased mitochondrial activity include diseases or disorders associated with mitochondrial dysfunction.

In certain embodiments, methods for treating diseases or disorders that would benefit from increased mitochondrial activity may comprise identifying a subject suffering from a mitochondrial dysfunction. Methods for diagnosing a mitochondrial dysfunction may involve molecular genetic, pathologic and/or biochemical analysis are summarized in Cohen and Gold, Cleveland Clinic Journal of Medicine, 68: 625-642 (2001). One method for diagnosing a mitochondrial dysfunction is the Thor-Byrne-ier scale (see e.g., Cohen and Gold, *supra*; Collin S. et al., Eur Neurol. 36: 260-267 (1996)). Other methods for determining mitochondrial number and function include, for example, enzymatic assays (e.g., a mitochondrial enzyme or an ATP biosynthesis factor such as an ETC enzyme or a

Krebs cycle enzyme), determination of mitochondrial mass, mitochondrial volume, and/or mitochondrial number, quantification of mitochondrial DNA, monitoring intracellular calcium homeostasis and/or cellular responses to perturbations of this homeostasis, evaluation of response to an apoptogenic stimulus, determination of
5 free radical production. Such methods are known in the art and are described, for example, in U.S. Patent Publication No. 2002/0049176 and references cited therein.

Mitochondria are critical for the survival and proper function of almost all types of eukaryotic cells. Mitochondria in virtually any cell type can have congenital or acquired defects that affect their function. Thus, the clinically significant signs
10 and symptoms of mitochondrial defects affecting respiratory chain function are heterogeneous and variable depending on the distribution of defective mitochondria among cells and the severity of their deficits, and upon physiological demands upon the affected cells. Nondividing tissues with high energy requirements, e.g. nervous tissue, skeletal muscle and cardiac muscle are particularly susceptible to
15 mitochondrial respiratory chain dysfunction, but any organ system can be affected.

Diseases and disorders associated with mitochondrial dysfunction include diseases and disorders in which deficits in mitochondrial respiratory chain activity contribute to the development of pathophysiology of such diseases or disorders in a mammal. This includes 1) congenital genetic deficiencies in activity of one or more
20 components of the mitochondrial respiratory chain; and 2) acquired deficiencies in the activity of one or more components of the mitochondrial respiratory chain, wherein such deficiencies are caused by a) oxidative damage during aging; b) elevated intracellular calcium; c) exposure of affected cells to nitric oxide; d) hypoxia or ischemia; e) microtubule-associated deficits in axonal transport of
25 mitochondria, or f) expression of mitochondrial uncoupling proteins.

Diseases or disorders that would benefit from increased mitochondrial activity generally include for example, diseases in which free radical mediated oxidative injury leads to tissue degeneration, diseases in which cells inappropriately undergo apoptosis, and diseases in which cells fail to undergo apoptosis. Exemplary
30 diseases or disorders that would benefit from increased mitochondrial activity include, for example, AD (Alzheimer's Disease), ADPD (Alzheimer's Disease and Parkinson's Disease), AMDF (Ataxia, Myoclonus and Deafness), auto-immune

disease, cancer, CIPO (Chronic Intestinal Pseudoobstruction with myopathy and Ophthalmoplegia), congenital muscular dystrophy, CPEO (Chronic Progressive External Ophthalmoplegia), DEAF (Maternally inherited DEAFness or aminoglycoside-induced DEAFness), DEMCHO (Dementia and Chorea), diabetes mellitus (Type I or Type II), DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness), DMDF (Diabetes Mellitus and Deafness), dystonia, Exercise Intolerance, ESOC (Epilepsy, Strokes, Optic atrophy, and Cognitive decline), FBSN (Familial Bilateral Striatal Necrosis), FICP (Fatal Infantile Cardiomyopathy Plus, a MELAS-associated cardiomyopathy), GER (Gastrointestinal Reflux), HD (Huntington's Disease), KSS (Kearns Sayre Syndrome), "later-onset" myopathy, LDYT (Leber's hereditary optic neuropathy and DYsTonia), Leigh's Syndrome, LHON (Leber Hereditary Optic Neuropathy), L IMM (Lethal Infantile Mitochondrial Myopathy), MDM (Myopathy and Diabetes Mellitus), MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes), MEPR (Myoclonic Epilepsy and Psychomotor Regression), MERME (MERRF/MELAS overlap disease), MERRF (Myoclonic Epilepsy and Ragged Red Muscle Fibers), MHCM (Maternally Inherited Hypertrophic CardioMyopathy), MICM (Maternally Inherited Cardiomyopathy), MILS (Maternally Inherited Leigh Syndrome), Mitochondrial Encephalocardiomyopathy, Mitochondrial Encephalomyopathy, MM (Mitochondrial Myopathy), MMC (Maternal Myopathy and Cardiomyopathy), MNGIE (Myopathy and external ophthalmoplegia, Neuropathy, Gastro-Intestinal, Encephalopathy), Multisystem Mitochondrial Disorder (myopathy, encephalopathy, blindness, hearing loss, peripheral neuropathy), NARP (Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa; alternate phenotype at this locus is reported as Leigh Disease), PD (Parkinson's Disease), Pearson's Syndrome, PEM (Progressive Encephalopathy), PEO (Progressive External Ophthalmoplegia), PME (Progressive Myoclonus Epilepsy), PMPS (Pearson Marrow-Pancreas Syndrome), psoriasis, RTT (Rett Syndrome), schizophrenia, SIDS (Sudden Infant Death Syndrome), SNHL (Sensorineural Hearing Loss), Varied Familial Presentation (clinical manifestations range from spastic paraparesis to multisystem progressive disorder & fatal cardiomyopathy to truncal ataxia, dysarthria, severe hearing loss, mental regression,

ptosis, ophthalmoparesis, distal cyclones, and diabetes mellitus), or Wolfram syndrome.

Other diseases and disorders that would benefit from increased mitochondrial activity include, for example, Friedreich's ataxia and other ataxias, amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, macular degeneration, epilepsy, Alpers syndrome, Multiple mitochondrial DNA deletion syndrome, MtDNA depletion syndrome, Complex I deficiency, Complex II (SDH) deficiency, Complex III deficiency, Cytochrome c oxidase (COX, Complex IV) deficiency, Complex V deficiency, Adenine Nucleotide Translocator (ANT) deficiency, Pyruvate dehydrogenase (PDH) deficiency, Ethylmalonic aciduria with lactic acidemia, 3-Methyl glutaconic aciduria with lactic acidemia, Refractory epilepsy with declines during infection, Asperger syndrome with declines during infection, Autism with declines during infection, Attention deficit hyperactivity disorder (ADHD), Cerebral palsy with declines during infection, Dyslexia with declines during infection, materially inherited thrombocytopenia and leukemia syndrome, MARIAHS syndrome (Mitochondrial ataxia, recurrent infections, aphasia, hypouricemia/hypomyelination, seizures, and dicarboxylic aciduria), ND6 dystonia, Cyclic vomiting syndrome with declines during infection, 3-Hydroxy isobutyric aciduria with lactic acidemia, Diabetes mellitus with lactic acidemia, Uridine responsive neurologic syndrome (URNS), Dilated cardiomyopathy, Splenic Lymphoma, and Renal Tubular Acidosis/Diabetes/Ataxis syndrome.

In other embodiments, the invention provides methods for treating a subject suffering from mitochondrial disorders arising from, but not limited to, post-traumatic head injury and cerebral edema, stroke (invention methods useful for preventing or preventing reperfusion injury), Lewy body dementia, hepatorenal syndrome, acute liver failure, NASH (non-alcoholic steatohepatitis), Anti-metastasis/prodifferentiation therapy of cancer, idiopathic congestive heart failure, atrial fibrillation (non-valvular), Wolff-Parkinson-White Syndrome, idiopathic heart block, prevention of reperfusion injury in acute myocardial infarctions, familial migraines, irritable bowel syndrome, secondary prevention of non-Q wave myocardial infarctions, Premenstrual syndrome, Prevention of renal failure in hepatorenal syndrome, anti-phospholipid antibody syndrome, eclampsia/pre-

eclampsia, oopause infertility, ischemic heart disease/angina, and Shy-Drager and unclassified dysautonomia syndromes.

In still another embodiment, there are provided methods for the treatment of mitochondrial disorders associated with pharmacological drug-related side effects.

- 5 Types of pharmaceutical agents that are associated with mitochondrial disorders include reverse transcriptase inhibitors, protease inhibitors, inhibitors of DHOD, and the like. Examples of reverse transcriptase inhibitors include, for example, Azidothymidine (AZT), Stavudine (D4T), Zalcitabine (ddC), Didanosine (DDI), Fluoriodoauracil (FIAU), Lamivudine (3TC), Abacavir and the like. Examples of
10 protease inhibitors include, for example, Ritonavir, Indinavir, Saquinavir, Nelfinavir and the like. Examples of inhibitors of dihydroorotate dehydrogenase (DHOD) include, for example, Leflunomide, Brequinar, and the like.

- Reverse transcriptase inhibitors not only inhibit reverse transcriptase but also polymerase gamma which is required for mitochondrial function. Inhibition of
15 polymerase gamma activity (e.g., with a reverse transcriptase inhibitor) therefore leads to mitochondrial dysfunction and/or a reduced mitochondrial mass which manifests itself in patients as hyperlactatemia. This type of condition may benefit from an increase in the number of mitochondria and/or an improvement in mitochondrial function, e.g., by administration of a sirtuin variant therapeutic agent.

- 20 Common symptoms of mitochondrial diseases include cardiomyopathy, muscle weakness and atrophy, developmental delays (involving motor, language, cognitive or executive function), ataxia, epilepsy, renal tubular acidosis, peripheral neuropathy, optic neuropathy, autonomic neuropathy, neurogenic bowel dysfunction, sensorineural deafness, neurogenic bladder dysfunction, dilating
25 cardiomyopathy, migraine, hepatic failure, lactic acidemia, and diabetes mellitus.

- In certain embodiments, the invention provides methods for treating a disease or disorder that would benefit from increased mitochondrial activity that involves administering to a subject in need thereof one or more sirtuin variant therapeutic agents in combination with another therapeutic agent such as, for
30 example, an agent useful for treating mitochondrial dysfunction (such as antioxidants, vitamins, or respiratory chain cofactors), an agent useful for reducing a symptom associated with a disease or disorder involving mitochondrial dysfunction

(such as, an anti-seizure agent, an agent useful for alleviating neuropathic pain, an agent for treating cardiac dysfunction), a cardiovascular agent (as described further below), a chemotherapeutic agent (as described further below), or an anti-neurodegeneration agent (as described further below). In an exemplary embodiment, the invention provides methods for treating a disease or disorder that would benefit from increased mitochondrial activity that involves administering to a subject in need thereof one or more sirtuin variant therapeutic agents in combination with one or more of the following: coenzyme Q₁₀, L-carnitine, thiamine, riboflavin, niacinamide, folate, vitamin E, selenium, lipoic acid, or prednisone. Compositions comprising such combinations are also provided herein.

In exemplary embodiments, the invention provides methods for treating diseases or disorders that would benefit from increased mitochondrial activity by administering to a subject a therapeutically effective amount of a sirtuin variant therapeutic agent. Exemplary diseases or disorders include, for example, neuromuscular disorders (e.g., Friedreich's Ataxia, muscular dystrophy, multiple sclerosis, etc.), disorders of neuronal instability (e.g., seizure disorders, migraine, etc.), developmental delay, neurodegenerative disorders (e.g., Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, etc.), ischemia, renal tubular acidosis, age-related neurodegeneration and cognitive decline, chemotherapy fatigue, age-related or chemotherapy-induced menopause or irregularities of menstrual cycling or ovulation, mitochondrial myopathies, mitochondrial damage (e.g., calcium accumulation, excitotoxicity, nitric oxide exposure, hypoxia, etc.), and mitochondrial deregulation.

A gene defect underlying Friedreich's Ataxia (FA), the most common hereditary ataxia, was recently identified and is designated "frataxin". In FA, after a period of normal development, deficits in coordination develop which progress to paralysis and death, typically between the ages of 30 and 40. The tissues affected most severely are the spinal cord, peripheral nerves, myocardium, and pancreas. Patients typically lose motor control and are confined to wheel chairs, and are commonly afflicted with heart failure and diabetes. The genetic basis for FA involves GAA trinucleotide repeats in an intron region of the gene encoding frataxin. The presence of these repeats results in reduced transcription and

expression of the gene. Frataxin is involved in regulation of mitochondrial iron content. When cellular frataxin content is subnormal, excess iron accumulates in mitochondria, promoting oxidative damage and consequent mitochondrial degeneration and dysfunction. When intermediate numbers of GAA repeats are
5 present in the frataxin gene intron, the severe clinical phenotype of ataxia may not develop. However, these intermediate-length trinucleotide extensions are found in 25 to 30% of patients with non-insulin dependent diabetes mellitus, compared to about 5% of the nondiabetic population. In certain embodiments, sirtuin variant therapeutic agents may be used for treating patients with disorders related to
10 deficiencies or defects in frataxin, including Friedreich's Ataxia, myocardial dysfunction, diabetes mellitus and complications of diabetes like peripheral neuropathy.

Muscular dystrophy refers to a family of diseases involving deterioration of neuromuscular structure and function, often resulting in atrophy of skeletal muscle
15 and myocardial dysfunction. In the case of Duchenne muscular dystrophy, mutations or deficits in a specific protein, dystrophin, are implicated in its etiology. Mice with their dystrophin genes inactivated display some characteristics of muscular dystrophy, and have an approximately 50% deficit in mitochondrial respiratory chain activity. A final common pathway for neuromuscular degeneration in most
20 cases is calcium-mediated impairment of mitochondrial function. In certain embodiments, sirtuin variant therapeutic agents may be used for reducing the rate of decline in muscular functional capacities and for improving muscular functional status in patients with muscular dystrophy.

Multiple sclerosis (MS) is a neuromuscular disease characterized by focal
25 inflammatory and autoimmune degeneration of cerebral white matter. Periodic exacerbations or attacks are significantly correlated with upper respiratory tract and other infections, both bacterial and viral, indicating that mitochondrial dysfunction plays a role in MS. Depression of neuronal mitochondrial respiratory chain activity caused by Nitric Oxide (produced by astrocytes and other cells involved in
30 inflammation) is implicated as a molecular mechanism contributing to MS. In certain embodiments, sirtuin variant therapeutic agents may be used for treatment of

patients with multiple sclerosis, both prophylactically and during episodes of disease exacerbation.

Epilepsy is often present in patients with mitochondrial cytopathies, involving a range of seizure severity and frequency, e.g. absence, tonic, atonic,
5 myoclonic, and status epilepticus, occurring in isolated episodes or many times daily. In certain embodiments, sirtuin variant therapeutic agents may be used for treating patients with seizures secondary to mitochondrial dysfunction, including reducing frequency and severity of seizure activity.

Metabolic studies on patients with recurrent migraine headaches indicate that
10 deficits in mitochondrial activity are commonly associated with this disorder, manifesting as impaired-oxidative phosphorylation and excess lactate production. Such deficits are not necessarily due to genetic defects in mitochondrial DNA. Migraineurs are hypersensitive to nitric oxide, an endogenous inhibitor of Cytochrome c Oxidase. In addition, patients with mitochondrial cytopathies, e.g.
15 MELAS, often have recurrent migraines. In certain embodiments, sirtuin variant therapeutic agents may be used for treating patients with recurrent migraine headaches, including headaches refractory to ergot compounds or serotonin receptor antagonists.

Delays in neurological or neuropsychological development are often found in
20 children with mitochondrial diseases. Development and remodeling of neural connections requires intensive biosynthetic activity, particularly involving synthesis of neuronal membranes and myelin, both of which require pyrimidine nucleotides as cofactors. Uridine nucleotides are involved inactivation and transfer of sugars to glycolipids and glycoproteins. Cytidine nucleotides are derived from uridine
25 nucleotides, and are crucial for synthesis of major membrane phospholipid constituents like phosphatidylcholine, which receives its choline moiety from cytidine diphosphocholine. In the case of mitochondrial dysfunction (due to either mitochondrial DNA defects or any of the acquired or conditional deficits like excitotoxic or nitric oxide-mediated mitochondrial dysfunction) or other conditions
30 resulting in impaired pyrimidine synthesis, cell proliferation and axonal extension is impaired at crucial stages in development of neuronal interconnections and circuits, resulting in delayed or arrested development of neuropsychological functions like

language, motor, social, executive function, and cognitive skills. In autism for example, magnetic resonance spectroscopy measurements of cerebral phosphate compounds indicates that there is global undersynthesis of membranes and membrane precursors indicated by reduced levels of uridine diphospho-sugars, and
5 cytidine nucleotide derivatives involved in membrane synthesis. Disorders characterized by developmental delay include Rett's Syndrome, pervasive developmental delay (or PDD-NOS "pervasive developmental delay not otherwise specified" to distinguish it from specific subcategories like autism), autism, Asperger's Syndrome, and Attention Deficit/Hyperactivity Disorder (ADHD), which
10 is becoming recognized as a delay or lag in development of neural circuitry underlying executive functions. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating treating patients with neurodevelopmental delays (e.g., involving motor, language, executive function, and cognitive skills), or other delays or arrests of neurological and neuropsychological development in the nervous
15 system and somatic development in non-neural tissues like muscle and endocrine glands.

The two most significant severe neurodegenerative diseases associated with aging, Alzheimer's Disease (AD) and Parkinson's Disease (PD), both involve mitochondrial dysfunction in their pathogenesis. Complex I deficiencies in particular
20 are frequently found not only in the nigrostriatal neurons that degenerate in Parkinson's disease, but also in peripheral tissues and cells like muscle and platelets of Parkinson's Disease patients. In Alzheimer's Disease, mitochondrial respiratory chain activity is often depressed, especially Complex IV (Cytochrome c Oxidase). Moreover, mitochondrial respiratory function altogether is depressed as a
25 consequence of aging, further amplifying the deleterious sequelae of additional molecular lesions affecting respiratory chain function. Other factors in addition to primary mitochondrial dysfunction underlie neurodegeneration in AD, PD, and related disorders. Excitotoxic stimulation and nitric oxide are implicated in both diseases, factors which both exacerbate mitochondrial respiratory chain deficits and
30 whose deleterious actions are exaggerated on a background of respiratory chain dysfunction. Huntington's Disease also involves mitochondrial dysfunction in affected brain regions, with cooperative interactions of excitotoxic stimulation and

mitochondrial dysfunction contributing to neuronal degeneration. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating and attenuating progression of age-related neurodegenerative diseases including AD and PD.

5 One of the major genetic defects in patients with Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's Disease) is mutation or deficiency in Copper-Zinc Superoxide Dismutase (SOD 1), an antioxidant enzyme. Mitochondria both produce and are primary targets for reactive oxygen species. Inefficient transfer of electrons to oxygen in mitochondria is the most significant physiological source of free
10 radicals in mammalian systems. Deficiencies in antioxidants or antioxidant enzymes can result in or exacerbate mitochondrial degeneration. Mice transgenic for mutated SOD1 develop symptoms and pathology similar to those in human ALS. The development of the disease in these animals has been shown to involve oxidative destruction of mitochondria followed by functional decline of motor neurons and
15 onset of clinical symptoms. Skeletal muscle from ALS patients has low mitochondrial Complex I activity. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating ALS, for reversing or slowing the progression of clinical symptoms.

 Oxygen deficiency results in both direct inhibition of mitochondrial
20 respiratory chain activity by depriving cells of a terminal electron acceptor for Cytochrome c reoxidation at Complex IV, and indirectly, especially in the nervous system, via secondary post-anoxic excitotoxicity and nitric oxide formation. In conditions like cerebral anoxia, angina or sickle cell anemia crises, tissues are relatively hypoxic. In such cases, treatments that increase mitochondrial activity
25 provide protection of affected tissues from deleterious effects of hypoxia, attenuate secondary delayed cell death, and accelerate recovery from hypoxic tissue stress and injury. In certain embodiments, sirtuin variant therapeutic agents may be useful for preventing delayed cell death (apoptosis in regions like the hippocampus or cortex occurring about 2 to 5 days after an episode of cerebral ischemia) after ischemic or
30 hypoxic insult to the brain.

 Acidosis due to renal dysfunction is often observed in patients with mitochondrial disease, whether the underlying respiratory chain dysfunction is

congenital or induced by ischemia or cytotoxic agents like cisplatin. Renal tubular acidosis often requires administration of exogenous sodium bicarbonate to maintain blood and tissue pH. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating renal tubular acidosis and other forms of renal dysfunction caused by mitochondrial respiratory chain deficits.

During normal aging, there is a progressive decline in mitochondrial respiratory chain function. Beginning about age 40, there is an exponential rise in accumulation of mitochondrial DNA defects in humans, and a concurrent decline in nuclear-regulated elements of mitochondrial respiratory activity. Many mitochondrial DNA lesions have a selection advantage during mitochondrial turnover, especially in postmitotic cells. The proposed mechanism is that mitochondria with a defective respiratory chain produce less oxidative damage to themselves than do mitochondria with intact functional respiratory chains (mitochondrial respiration is the primary source of free radicals in the body). Therefore, normally-functioning mitochondria accumulate oxidative damage to membrane lipids more rapidly than do defective mitochondria, and are therefore "tagged" for degradation by lysosomes. Since mitochondria within cells have a half life of about 10 days, a selection advantage can result in rapid replacement of functional mitochondria with those with diminished respiratory activity, especially in slowly dividing cells. The net result is that once a mutation in a gene for a mitochondrial protein that reduces oxidative damage to mitochondria occurs, such defective mitochondria will rapidly populate the cell, diminishing or eliminating its respiratory capabilities. The accumulation of such cells results in aging or degenerative disease at the organismal level. This is consistent with the progressive mosaic appearance of cells with defective electron transport activity in muscle, with cells almost devoid of Cytochrome c Oxidase (COX) activity interspersed randomly amidst cells with normal activity, and a higher incidence of COX-negative cells in biopsies from older subjects. The organism, during aging, or in a variety of mitochondrial diseases, is thus faced with a situation in which irreplaceable postmitotic cells (e.g. neurons, skeletal and cardiac muscle) must be preserved and their function maintained to a significant degree, in the face of an inexorable progressive decline in mitochondrial respiratory chain function. Neurons with

dysfunctional mitochondria become progressively more sensitive to insults like excitotoxic injury. Mitochondrial failure contributes to most degenerative diseases (especially neurodegeneration) that accompany aging. Congenital mitochondrial diseases often involve early-onset neurodegeneration similar in fundamental
5 mechanism to disorders that occur during aging of people born with normal mitochondria. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating or attenuating cognitive decline and other degenerative consequences of aging.

Mitochondrial DNA damage is more extensive and persists longer than
10 nuclear DNA damage in cells subjected to oxidative stress or cancer chemotherapy agents like cisplatin due to both greater vulnerability and less efficient repair of mitochondrial DNA. Although mitochondrial DNA may be more sensitive to damage than nuclear DNA, it is relatively resistant, in some situations, to mutagenesis by chemical carcinogens. This is because mitochondria respond to
15 some types of mitochondrial DNA damage by destroying their defective genomes rather than attempting to repair them. This results in global mitochondrial dysfunction for a period after cytotoxic chemotherapy. Clinical use of chemotherapy agents like cisplatin, mitomycin, and cytoxan is often accompanied by debilitating "chemotherapy fatigue", prolonged periods of weakness and exercise intolerance
20 which may persist even after recovery from hematologic and gastrointestinal toxicities of such agents. In certain embodiments, sirtuin variant therapeutic agents may be useful for treatment and prevention of side effects of cancer chemotherapy related to mitochondrial dysfunction.

A crucial function of the ovary is to maintain integrity of the mitochondrial
25 genome in oocytes, since mitochondria passed onto a fetus are all derived from those present in oocytes at the time of conception. Deletions in mitochondrial DNA become detectable around the age of menopause, and are also associated with abnormal menstrual cycles. Since cells cannot directly detect and respond to defects in mitochondrial DNA, but can only detect secondary effects that affect the
30 cytoplasm, like impaired respiration, redox status, or deficits in pyrimidine synthesis, such products of mitochondrial function participate as a signal for oocyte selection and follicular atresia, ultimately triggering menopause when maintenance

of mitochondrial genomic fidelity and functional activity can no longer be guaranteed. This is analogous to apoptosis in cells with DNA damage, which undergo an active process of cellular suicide when genomic fidelity can no longer be achieved by repair processes. Women with mitochondrial cytopathies affecting the gonads often undergo premature menopause or display primary cycling abnormalities. Cytotoxic cancer chemotherapy often induces premature menopause, with a consequent increased risk of osteoporosis. Chemotherapy-induced amenorrhea is generally due to primary ovarian failure. The incidence of chemotherapy-induced amenorrhea increases as a function of age in premenopausal women receiving chemotherapy, pointing toward mitochondrial involvement. Inhibitors of mitochondrial respiration or protein synthesis inhibit hormone-induced ovulation, and furthermore inhibit production of ovarian steroid hormones in response to pituitary gonadotropins. Women with Down's syndrome typically undergo menopause prematurely, and also are subject to early onset of Alzheimer-like dementia. Low activity of cytochrome oxidase is consistently found in tissues of Down's patients and in late-onset Alzheimer's Disease. Appropriate support of mitochondrial function or compensation for mitochondrial dysfunction therefore is useful for protecting against age-related or chemotherapy-induced menopause or irregularities of menstrual cycling or ovulation. In certain embodiments, sirtuin variant therapeutic agents may be useful for treating and preventing amenorrhea, irregular ovulation, menopause, or secondary consequences of menopause.

In certain embodiments, sirtuin variant therapeutic agents may be useful for treatment mitochondrial myopathies. Mitochondrial myopathies range from mild, slowly progressive weakness of the extraocular muscles to severe, fatal infantile myopathies and multisystem encephalomyopathies. Some syndromes have been defined, with some overlap between them. Established syndromes affecting muscle include progressive external ophthalmoplegia, the Kearns-Sayre syndrome (with ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects, cerebellar ataxia, and sensorineural deafness), the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), the MERFF syndrome (myoclonic epilepsy and ragged red fibers), limb-girdle distribution weakness, and infantile myopathy (benign or severe and fatal). Muscle biopsy

specimens stained with modified Gomori's trichrome stain show ragged red fibers due to excessive accumulation of mitochondria. Biochemical defects in substrate transport and utilization, the Krebs cycle, oxidative phosphorylation, or the respiratory chain are detectable. Numerous mitochondrial DNA point mutations and
5 deletions have been described, transmitted in a maternal, nonmendelian inheritance pattern. Mutations in nuclear-encoded mitochondrial enzymes occur.

In certain embodiments, sirtuin variant therapeutic agents may be useful for treating patients suffering from toxic damage to mitochondria, such as, toxic damage due to calcium accumulation, excitotoxicity, nitric oxide exposure, drug induced
10 toxic damage, or hypoxia.

A fundamental mechanism of cell injury, especially in excitable tissues, involves excessive calcium entry into cells, as a result of either leakage through the plasma membrane or defects in intracellular calcium handling mechanisms. Mitochondria are major sites of calcium sequestration, and preferentially utilize
15 energy from the respiratory chain for taking up calcium rather than for ATP synthesis, which results in a downward spiral of mitochondrial failure, since calcium uptake into mitochondria results in diminished capabilities for energy transduction.

Excessive stimulation of neurons with excitatory amino acids is a common mechanism of cell death or injury in the central nervous system. Activation of
20 glutamate receptors, especially of the subtype designated NMDA receptors, results in mitochondrial dysfunction, in part through elevation of intracellular calcium during excitotoxic stimulation. Conversely, deficits in mitochondrial respiration and oxidative phosphorylation sensitizes cells to excitotoxic stimuli, resulting in cell death or injury during exposure to levels of excitotoxic neurotransmitters or toxins
25 that would be innocuous to normal cells.

Nitric oxide (about 1 micromolar) inhibits cytochrome oxidase (Complex IV) and thereby inhibits mitochondrial respiration; moreover, prolonged exposure to nitric oxide (NO) irreversibly reduces Complex I activity. Physiological or pathophysiological concentrations of NO thereby inhibit pyrimidine biosynthesis.
30 Nitric oxide is implicated in a variety of neurodegenerative disorders including inflammatory and autoimmune diseases of the central nervous system, and is involved in mediation of excitotoxic and post-hypoxic damage to neurons.

Oxygen is the terminal electron acceptor in the respiratory chain. Oxygen deficiency impairs electron transport chain activity, resulting in diminished pyrimidine synthesis as well as diminished ATP synthesis via oxidative phosphorylation. Human cells proliferate and retain viability under virtually anaerobic conditions if provided with uridine and pyruvate (or a similarly effective agent for oxidizing NADH to optimize glycolytic ATP production).

In certain embodiments, sirtuin variant therapeutic agents may be useful for treating diseases or disorders associated with mitochondrial deregulation.

Transcription of mitochondrial DNA encoding respiratory chain components requires nuclear factors. In neuronal axons, mitochondria must shuttle back and forth to the nucleus in order to maintain respiratory chain activity. If axonal transport is impaired by hypoxia or by drugs like taxol which affect microtubule stability, mitochondria distant from the nucleus undergo loss of cytochrome oxidase activity. Accordingly, treatment with a sirtuin variant therapeutic agent may be useful for promoting nuclear-mitochondrial interactions.

Mitochondria are the primary source of free radicals and reactive oxygen species, due to spillover from the mitochondrial respiratory chain, especially when defects in one or more respiratory chain components impairs orderly transfer of electrons from metabolic intermediates to molecular oxygen. To reduce oxidative damage, cells can compensate by expressing mitochondrial uncoupling proteins (UCP), of which several have been identified. UCP-2 is transcribed in response to oxidative damage, inflammatory cytokines, or excess lipid loads, e.g. fatty liver and steatohepatitis. UCPs reduce spillover of reactive oxygen species from mitochondria by discharging proton gradients across the mitochondrial inner membrane, in effect wasting energy produced by metabolism and rendering cells vulnerable to energy stress as a trade-off for reduced oxidative injury.

Muscle Performance

In other embodiments, the invention provides methods for enhancing muscle performance by administering a therapeutically effective amount of a sirtuin variant therapeutic agent. For example, sirtuin variant therapeutic agents may be useful for improving physical endurance (e.g., ability to perform a physical task such as exercise, physical labor, sports activities, etc.), inhibiting or retarding physical

fatigues, enhancing blood oxygen levels, enhancing energy in healthy individuals, enhance working capacity and endurance, reducing muscle fatigue, reducing stress, enhancing cardiac and cardiovascular function, improving sexual ability, increasing muscle ATP levels, and/or reducing lactic acid in blood. In certain embodiments, the methods involve administering an amount of a sirtuin variant therapeutic agent that increase mitochondrial activity, increase mitochondrial biogenesis, and/or increase mitochondrial mass.

Sports performance refers to the ability of the athlete's muscles to perform when participating in sports activities. Enhanced sports performance, strength, speed and endurance are measured by an increase in muscular contraction strength, increase in amplitude of muscle contraction, shortening of muscle reaction time between stimulation and contraction. Athlete refers to an individual who participates in sports at any level and who seeks to achieve an improved level of strength, speed and endurance in their performance, such as, for example, body builders, bicyclists, long distance runners, short distance runners, etc. An athlete may be hard training, that is, performs sports activities intensely more than three days a week or for competition. An athlete may also be a fitness enthusiast who seeks to improve general health and well-being, improve energy levels, who works out for about 1-2 hours about 3 times a week. Enhanced sports performance is manifested by the ability to overcome muscle fatigue, ability to maintain activity for longer periods of time, and have a more effective workout.

In the arena of athlete muscle performance, it is desirable to create conditions that permit competition or training at higher levels of resistance for a prolonged period of time. However, acute and intense anaerobic use of skeletal muscles often results in impaired athletic performance, with losses in force and work output, and increased onset of muscle fatigue, soreness, and dysfunction. It is now recognized that even a single exhaustive exercise session, or for that matter any acute trauma to the body such as muscle injury, resistance or exhaustive muscle exercise, or elective surgery, is characterized by perturbed metabolism that affects muscle performance in both short and long term phases. Both muscle metabolic/enzymatic activity and gene expression are affected. For example, disruption of skeletal muscle nitrogen metabolism as well as depletion of sources of metabolic energy occur during

extensive muscle activity. Amino acids, including branched-chain amino acids, are released from muscles followed by their deamination to elevate serum ammonia and local oxidation as muscle fuel sources, which augments metabolic acidosis. In addition, there is a decline in catalytic efficiency of muscle contraction events, as well as an alteration of enzymatic activities of nitrogen and energy metabolism. Further, protein catabolism is initiated where rate of protein synthesis is decreased coupled with an increase in the degradation of non-contractible protein. These metabolic processes are also accompanied by free radical generation which further damages muscle cells.

Recovery from fatigue during acute and extended exercise requires reversal of metabolic and non-metabolic fatiguing factors. Known factors that participate in human muscle fatigue, such as lactate, ammonia, hydrogen ion, etc., provide an incomplete and unsatisfactory explanation of the fatigue/recovery process, and it is likely that additional unknown agents participate (Baker et al., *J. Appl. Physiol.* 74:2294-2300, 1993; Bazzarre et al., *J Am. Coll. Nutr.* 11:505-511, 1992; Dohm et al., *Fed. Proc.* 44:348-352, 1985; Edwards In: *Biochemistry of Exercise, Proceedings of the Fifth International Symposium on the Biochemistry of Exercise* (Kutrgen, Vogel, Poormans, eds.), 1983; MacDougall et al., *Acta Physiol. Scand.* 146:403-404, 1992; Walser et al., *Kidney Int.* 32:123-128, 1987). Several studies have also analyzed the effects of nutritional supplements and herbal supplements in enhancing muscle performance.

Aside from muscle performance during endurance exercise, free radicals and oxidative stress parameters are affected in pathophysiological states. A substantial body of data now suggests that oxidative stress contributes to muscle wasting or atrophy in pathophysiological states (reviewed in Clarkson, P. M. *Antioxidants and physical performance. Crit. Rev. Food Sci. Nutr.* 35: 31-41; 1995; Powers, S. K.; Lennon, S. L. *Analysis of cellular responses to free radicals: Focus on exercise and skeletal muscle. Proc. Nutr. Soc.* 58: 1025-1033; 1999). For example, with respect to muscular disorders where both muscle endurance and function are compensated, the role of nitric oxide (NO), has been implicated. In muscular dystrophies, especially those due to defects in proteins that make up the dystrophin-glycoprotein complex (DGC), the enzyme that synthesizes NO, nitric oxide synthase (NOS), has been

associated. Recent studies of dystrophies related to DGC defects suggest that one mechanism of cellular injury is functional ischemia related to alterations in cellular NOS and disruption of a normal protective action of NO. This protective action is the prevention of local ischemia during contraction-induced increases in sympathetic vasoconstriction. Rando (Microsc Res Tech 55(4):223-35, 2001), has shown that oxidative injury precedes pathologic changes and that muscle cells with defects in the DGC have an increased susceptibility to oxidant challenges. Excessive lipid peroxidation due to free radicals has also been shown to be a factor in myopathic diseases such as McArdle's disease (Russo et al., Med Hypotheses. 39(2):147-51, 1992). Furthermore, mitochondrial dysfunction is a well-known correlate of age-related muscle wasting (sarcopenia) and free radical damage has been suggested, though poorly investigated, as a contributing factor (reviewed in Navarro, A.; Lopez-Cepero, J. M.; Sanchez del Pino, M. L. Front. Biosci. 6: D26-44; 2001). Other indications include acute sarcopenia, for example muscle atrophy and/or cachexia associated with burns, bed rest, limb immobilization, or major thoracic, abdominal, and/or orthopedic surgery. It is contemplated that sirtuin variant therapeutic agents will also be effective in the treatment of muscle related pathological conditions.

Other Uses

Sirtuin variant therapeutic agents may be used for treating or preventing viral infections (such as infections by influenza, herpes or papilloma virus) or as antifungal agents. In certain embodiments, sirtuin variant therapeutic agents may be administered as part of a combination drug therapy with another therapeutic agent for the treatment of viral diseases, including, for example, acyclovir, ganciclovir and zidovudine. In another embodiment, sirtuin variant therapeutic agents may be administered as part of a combination drug therapy with another anti-fungal agent including, for example, topical anti-fungals such as ciclopirox, clotrimazole, econazole, miconazole, nystatin, oxiconazole, terconazole, and tolnaftate, or systemic anti-fungal such as fluconazole (Diflucan), itraconazole (Sporanox), ketoconazole (Nizoral), and miconazole (Monistat I.V.).

Subjects that may be treated as described herein include eukaryotes, such as mammals, e.g., humans, ovines, bovines, equines, porcines, canines, felines, non-

human primate, mice, and rats. Cells that may be treated include eukaryotic cells, e.g., from a subject described above, or plant cells, yeast cells and prokaryotic cells, e.g., bacterial cells. For example, sirtuin variant therapeutic agents may be administered to farm animals to improve their ability to withstand farming conditions longer.

Sirtuin variant therapeutic agents may also be used to increase lifespan, stress resistance, and resistance to apoptosis in plants. In one embodiment, a sirtuin variant therapeutic agent is introduced into plants or to fungi. In another embodiment, plants are genetically modified to produce a sirtuin variant. In another embodiment, plants and fruits are treated with a sirtuin variant prior to picking and shipping to increase resistance to damage during shipping. Plant seeds may also be contacted with sirtuin variants described herein, e.g., to preserve them.

In other embodiments, sirtuin variant therapeutic agents may be used for modulating lifespan in yeast cells. Situations in which it may be desirable to extend the lifespan of yeast cells include any process in which yeast is used, e.g., the making of beer, yogurt, and bakery items, e.g., bread. Use of yeast having an extended lifespan can result in using less yeast or in having the yeast be active for longer periods of time. Yeast or other mammalian cells used for recombinantly producing proteins may also be treated as described herein.

Sirtuin variant therapeutic agents may also be used to increase lifespan, stress resistance and resistance to apoptosis in insects. In this embodiment, sirtuin variants would be applied to useful insects, e.g., bees and other insects that are involved in pollination of plants. In a specific embodiment, a sirtuin variant would be applied to bees involved in the production of honey. Generally, the methods described herein may be applied to any organism, e.g., eukaryote, that may have commercial importance. For example, they can be applied to fish (aquaculture) and birds (e.g., chicken and fowl).

At least in view of the link between reproduction and longevity (Longo and Finch, Science, 2002), sirtuin variant therapeutic agents can be applied to affect the reproduction of organisms such as insects, animals and microorganisms.

7. Pharmaceutical Compositions

The sirtuin variant therapeutic agents described herein may be used alone, or as part of a conjoint therapy with other compounds/pharmaceutical compositions.

Sirtuin variant therapeutic agents may be conveniently formulated for administration with a biologically acceptable medium, such as water, buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like) or suitable mixtures thereof. The optimum concentration of the sirtuin variant therapeutic agent(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists. As used herein, "biologically acceptable medium" includes any and all solvents, dispersion media, and the like which may be appropriate for the desired route of administration of the pharmaceutical preparation. The use of such media for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the activity of the therapeutics, its use in the pharmaceutical preparation of the invention is contemplated. Suitable vehicles and their formulation inclusive of other proteins are described, for example, in the book Remington's Pharmaceutical Sciences. (Remington's Pharmaceutical Sciences. Mack Publishing Co., Easton, Pa., USA 1985). These vehicles include injectable "deposit formulations."

Pharmaceutical formulations of the present invention can also include veterinary compositions, e.g., pharmaceutical preparations of sirtuin variant therapeutic agents suitable for veterinary uses, e.g., for the treatment of live stock (cow, sheep, goat, pig, and horse, etc.) or domestic animals, e.g., cats and dogs.

Sirtuin variant therapeutic agents may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinacious biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a therapeutic at a particular target site.

Sustained-release preparations are also provided herein. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing a sirtuin variant therapeutic agent, which matrices are in the

form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable
5 ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time
10 periods.

The pharmaceutical compositions according to the present invention may be administered as either a single dose or in multiple doses. The pharmaceutical compositions of the present invention may be administered either as individual therapeutic agents or in combination with other therapeutic agents. The treatments of
15 the present invention may be combined with conventional therapies, which may be administered sequentially or simultaneously. The pharmaceutical compositions of the present invention may be administered by any means that enables the sirtuin variant therapeutic agents to reach the targeted cells/tissues/organs. In some embodiments, routes of administration include those selected from the group
20 consisting of oral, intravesically, intravenous, intraarterial, intraperitoneal, local administration into the blood supply of the organ in which the targeted cells reside or directly into the cells. Intravenous administration is the preferred mode of administration. It may be accomplished with the aid of an infusion pump.

Sirtuin variant therapeutic agents may be administered to humans and other
25 animals for therapy by any suitable route of administration, including orally, intravesically, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the sirtuin variant
30 therapeutic agents of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are

formulated into pharmaceutically acceptable dosage forms such as described below or by other conventional methods known to those of skill in the art.

Actual dosage levels of the sirtuin variant therapeutic agents in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular sirtuin variant therapeutic agent employed, the route of administration, the time of administration, the rate of excretion of the particular polypeptide being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular therapeutic employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the sirtuin variant therapeutic agent employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a sirtuin variant therapeutic agent will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

If desired, the effective daily dose of the sirtuin variant therapeutic agent may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

The patient receiving this treatment may be any animal in need, including primates, in particular humans, and other non-human mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

5 Sirtuin variant therapeutic agents can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with other therapeutic agents as described herein. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active agents in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

10 Cells, e.g., treated *ex vivo* with a sirtuin variant therapeutic agent, can be administered according to methods for administering a graft to a subject, which may be accompanied, e.g., by administration of an immunosuppressant drug, e.g., cyclosporin A. For general principles in medicinal formulation, the reader is referred to Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular
15 Immunotherapy, by G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996; and Hematopoietic Stem Cell Therapy, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000.

 In certain embodiments, the methods described herein involve administering to a subject nucleic acid encoding a sirtuin variant. Delivery of nucleic acids may
20 achieved using *in vivo* or *ex vivo* gene therapy methods. For *in vivo* gene therapy approaches, expression constructs of the therapeutic sirtuin variants may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively transfecting cells *in vivo* with a recombinant fusion gene. Approaches include insertion of the subject fusion gene in viral vectors
25 including recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors can be used to transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized liposomes (e.g. antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other
30 such intracellular carriers, as well as direct injection of the gene construct or CaPO₄ precipitation carried out *in vivo*. It will be appreciated that because transduction of appropriate target cells represents the critical first step in gene therapy, choice of the

particular gene delivery system will depend on such factors as the phenotype of the intended target and the route of administration, *e.g.* locally or systemically. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery system of choice for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. A major prerequisite for the use of retroviruses is to ensure the safety of their use, particularly with regard to the possibility of the spread of wild-type virus in the cell population. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:271). Thus, recombinant retrovirus can be constructed in which part of the retroviral coding sequence (gag, pol, env) has been replaced by nucleic acid encoding a CKI polypeptide, rendering the retrovirus replication defective. The replication defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in *Current Protocols in Molecular Biology*, Ausubel, F.M. *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include ψ Crip, ψ Cre, ψ 2 and ψ Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including neural cells, epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, *in vitro* and/or *in vivo* (see for example Eglitis, *et al.* (1985) *Science* 230:1395-1; Danos and

Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.* (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381;
5 Chowdhury *et al.* (1991) Science 254:1802-1805; van Beusechem *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay *et al.* (1992) Human Gene Therapy 3:641-647; Dai *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu *et al.* (1993) J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT
10 Application WO 89/05345; and PCT Application WO 92/07573).

Furthermore, it has been shown that it is possible to limit the infection spectrum of retroviruses and consequently of retroviral-based vectors, by modifying the viral packaging polypeptides on the surface of the viral particle (see, for example PCT publications WO93/25234, WO94/06920, and WO94/11524). For instance,
15 strategies for the modification of the infection spectrum of retroviral vectors include: coupling antibodies specific for cell surface antigens to the viral env polypeptide (Roux *et al.* (1989) PNAS 86:9079-9083; Julan *et al.* (1992) J. Gen Virol 73:3251-3255; and Goud *et al.* (1983) Virology 163:251-254); or coupling cell surface ligands to the viral env polypeptides (Neda *et al.* (1991) J Biol Chem 266:14143-
20 14146). Coupling can be in the form of the chemical cross-linking with a polypeptide or other variety (*e.g.* lactose to convert the env polypeptide to an asialoglycopolypeptide), as well as by generating fusion polypeptides (*e.g.* single-chain antibody/env fusion polypeptides). This technique, while useful to limit or otherwise direct the infection to certain tissue types, and can also be used to convert
25 an ecotropic vector in to an amphotropic vector.

In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be employed to cause expression of the subject sirtuin variants in the tissue of an animal. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular
30 transport of macromolecules. In preferred embodiments, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the gene by the targeted cell. Exemplary gene delivery systems of this type include

liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In a representative embodiment, a gene encoding one of the SIRT1 variants can be entrapped in liposomes bearing positive charges on their surface (*e.g.*, lipofectins) and (optionally) which are tagged with antibodies against cell surface
5 antigens of the target tissue (Mizuno *et al.* (1992) No Shinkei Geka 20:547-551; PCT publication WO91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075). For example, lipofection of neuroglion^{***} cells can be carried out using liposomes tagged with monoclonal antibodies against glioma-associated antigen (Mizuno *et al.* (1992) Neurol. Med. Chir. 32:873-876).

10 In clinical settings, the gene delivery systems can be introduced into a patient by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, *e.g.* by intravenous injection, and specific transduction of the target cells occurs predominantly from specificity of transfection provided by
15 the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent
20 5,328,470) or by stereotactic injection (*e.g.* Chen *et al.* (1994) PNAS 91: 3054-3057).

Toxicity and therapeutic efficacy of sirtuin variant therapeutic agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The LD₅₀ is the dose lethal to 50% of the population. The ED₅₀ is the
25 dose of a drug which produces 50% maximum response or effect, or alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations. The dose ratio between toxic and therapeutic effects (LD₅₀/ED₅₀) is the therapeutic index. Sirtuin variant therapeutic agents that exhibit large therapeutic indexes are preferred. While sirtuin variant therapeutic agents that
30 exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such agents may lie within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form
5 employed and the route of administration utilized. For any agent, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such
10 information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

8. Kits

Also provided herein are kits, e.g., kits for therapeutic purposes or kits for modulating the lifespan of cells or modulating apoptosis. A kit may comprise one or more sirtuin variant therapeutic agents, e.g., in premeasured doses. A kit may optionally comprise devices for contacting cells with the compounds and instructions for use. Devices include syringes, stents and other devices for
20 introducing a sirtuin variant therapeutic agent into a subject (e.g., the blood vessel of a subject) or applying it to the skin of a subject.

Another type of kit contemplated by the invention are kits for identifying sirtuin-modulating compounds. Such kits contain (1) a sirtuin variant polypeptide or a nucleic acid encoding a sirtuin variant polypeptide and, optionally, (2) a
25 sirtuin-modulating compound, for use as a control. The reagents may be in separate vessels. Such kits can be used, for example, to perform assays to test other compounds (typically provided by the user) for sirtuin-modulating activity. In certain embodiments, these kits further comprise means for determining sirtuin activity (e.g., a peptide with an appropriate indicator, such as those disclosed
30 herein).

The practice of the present methods will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic

biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

9. Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention in any way.

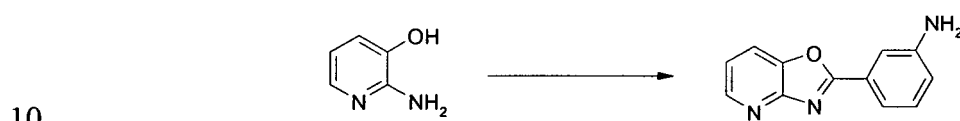
25 **EXAMPLE 1: SIRT1 Protein Expression**

Human SIRT1 constructs were expressed from the vectors which place expression under the control of the T7 promoter. The protein was expressed in E. coli BL21 Star (DE3) (Invitrogen) for both His and GST tagged proteins in LB media at 37 °C until OD600 reaching 0.8. The temperature of the culture was cooled down to 16 °C on ice and IPTG was added to 1 mM. The culture was incubated at 16 °C for 14-16 hrs and cells were harvest by centrifugation at 29,000 g for 30 min at 4 °C. For higher purity, protein was purified by Ni²⁺-chelate chromatography.

The eluted protein was then purified by ion exchange chromatography, followed by size exclusion chromatography. The resulting protein was typically >95% pure as assessed by SDS-PAGE analysis. SIRT1-G5c, H5c, I5c, J5c, K5c, L5c, M5c, N5c were purified by Ni²⁺-chelate chromatography only. SIRT1 truncations, A8, B8, C8, D8, and E8 were generated by Sareum Ltd. The protein concentration was measured using Biorad Protein Assay with bovine gamma globulin as standard.

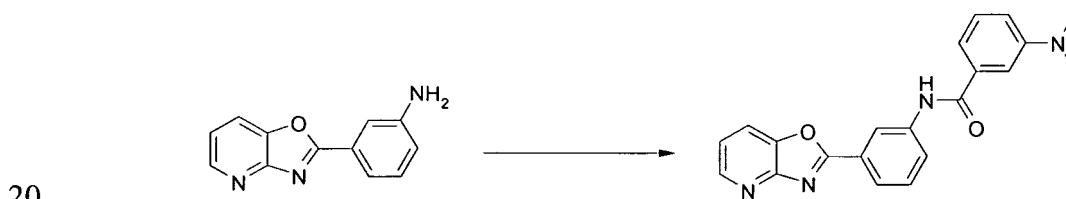
EXAMPLE 2: Compound Synthesis

Preparation of 3-(oxazolo[4,5-b]pyridin-2-yl)benzenamine:



A 50 mL flask was charged with 2-amino-3-hydroxypyridine (1.0 g, 9.1 mmol), 3-aminobenzoic acid (1.24 g, 9.1 mmol) and polyphosphoric acid (12 mL). The reaction mixture was stirred with heating (200 °C x 4h). The mixture was then cooled slightly and quenched with ice-water (150 mL). The mixture was neutralized to pH 7.5 with sat. Na₂CO₃. The product was extracted with EtOAc, and the combined organic extracts were washed with brine and dried (MgSO₄). Solvent evaporation afforded 3-oxazolo[4,5-b]pyridin-2-yl-benzenamine. (Calc'd for C₁₂H₉N₃O: 211.2, [M+H]⁺ found: 212)

Preparation of Compound #1

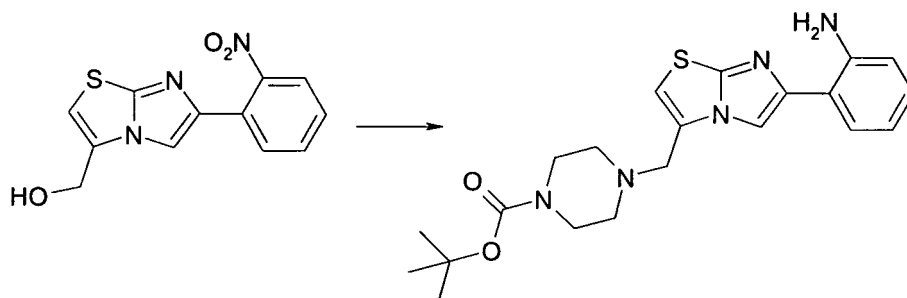


To a solution of the aniline (0.375g, 1.8 mmol) in DMF (6 mL) was added 3-dimethylaminobenzoic acid (0.29 g, 1.8 mmol), HATU (1.0 g, 2.7 mmol), HOAt (0.36 g, 2.7 mmol) and diisopropylethylamine (0.69 g, 5.3 mmol). The reaction mixture was stirred with heating (70 °C x 12 h). After dilution with CH₂Cl₂, the organic layer was washed with sat. NaHCO₃ and brine, and dried (MgSO₄). The crude material was purified by silica chromatography (0-10% MeOH/CH₂Cl₂) to

afford 3-dimethylamino-N-(3-oxazolo[4,5-b]pyridin-2-yl-phenyl)-benzamide.
(Calc'd for C₂₁H₁₈N₄O₂: 358, [M+H]⁺ found: 359)

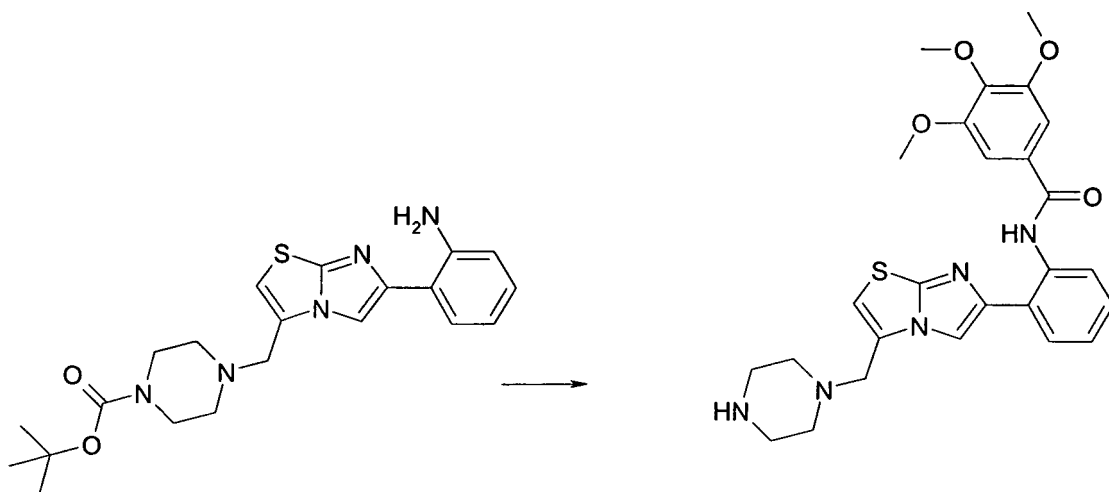
Preparation of 4-[6-(2-amino-phenyl)-imidazo[2,1-b]thiazol-3-ylmethyl]-
piperazine-1-carboxylic acid tert-butyl ester:

5



- [6-(2-Nitro-phenyl)-imidazo[2,1-b]thiazol-3-yl]-methanol (1.0 g, 3.64 mmol) was dissolved in CH₂Cl₂ (100 mL) along with Et₃N (0.51 mL, 3.64 mmol).
10 Methanesulfonyl chloride (1 eq, 0.28 mL) was added and the reaction mixture was warmed to room temperature and stirred for 15 min. It was then quenched with brine and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄) and concentrated to afford the mesylate intermediate. This material was taken up CH₃CN (4 mL) along with Et₃N (0.51 mL, 3.64 mmol) and Boc-piperazine (680 mg,
15 3.64 mmol) and stirred at room temperature for 1 day. The reaction mixture was concentrated and the resulting residue was partitioned between CH₂Cl₂ and water. The organic layer was dried (Na₂SO₄) and concentrated to afford essentially quantitative yield of the product. This material was taken up in MeOH (6 mL) and water (1 mL) along with sodium hydrosulfide hydrate (200 mg). The resulting
20 reaction mixture was stirred under reflux for 24 hours. It was then cooled to room temperature and concentrated. The resulting residue was diluted with water (2 mL) and extracted with CH₂Cl₂. The combined organic layers were dried (Na₂SO₄) and concentrated to afford 0.90 g of 4-[6-(2-amino-phenyl)-imidazo[2,1-b]thiazol-3-ylmethyl]-piperazine-1-carboxylic acid tert-butyl ester (Calc'd for C₂₁H₂₇N₅O₂S:
25 413.5, [M+H]⁺ found: 414).

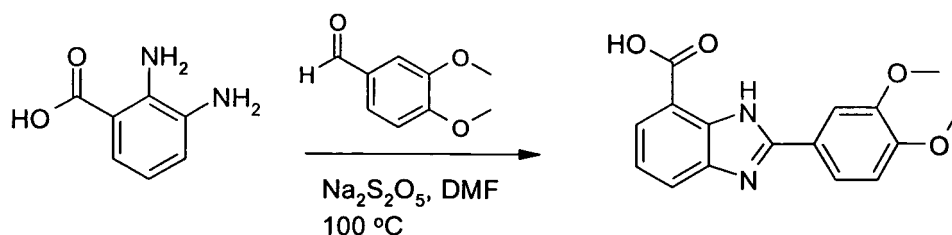
Preparation of Compound #2:



4-[6-(2-Amino-phenyl)-imidazo[2,1-b]thiazol-3-ylmethyl]-piperazine-1-carboxylic acid tert-butyl ester (0.3 mmol) was taken up in 1 mL of pyridine along with 1 eq (67 mg) of 3,4,5-trimethoxybenzoyl chloride. The reaction mixture was heated in a Biotage microwave reactor (160° x10 min). It was then cooled to room temperature and concentrated. The resulting crude product was purified by chromatography (Isco, gradient elution, CH₂Cl₂ to 95% CH₂Cl₂, 4% MeOH and 1% Et₃N). The purified product was then treated with a solution containing 25% TFA in CH₂Cl₂ (2mL) for 2 hours. It was then concentrated and the resulting residue was triturated with Et₂O to afford the desired product as the TFA salt (Calc'd for C₂₆H₂₉N₅O₄S: 507.6, [M+H]⁺ found: 508).

¹HNMR (300 MHz, DMSO-d₆) δ: 9.9 (br s, 1 H), 9.0 (br s, 1 H), 8.7-7.10 (m, 7 H), 8.5 (s, 1 H), 4.0 (br s, 9 H), 3.8 (m, 2 H), 3.2-2.8 (m, 8 H). The analytical HPLC was performed on an Agilent 1100 Series HPLC equipped with a 3.5 μm Eclipse XDB-C18 (4.6 mm x 100 mm) column with the following conditions: MeCN/H₂O, modified with 0.1 % Formic acid mobile phase. Gradient elution: 5% hold (2 min), 5% to 95% gradient (11 min), 95% to 5% gradient (0.3 min), 5% hold (2.7 min), 15 min. total run time. Flow rate: 0.8 ml/min. Retention time = 3.77 min.

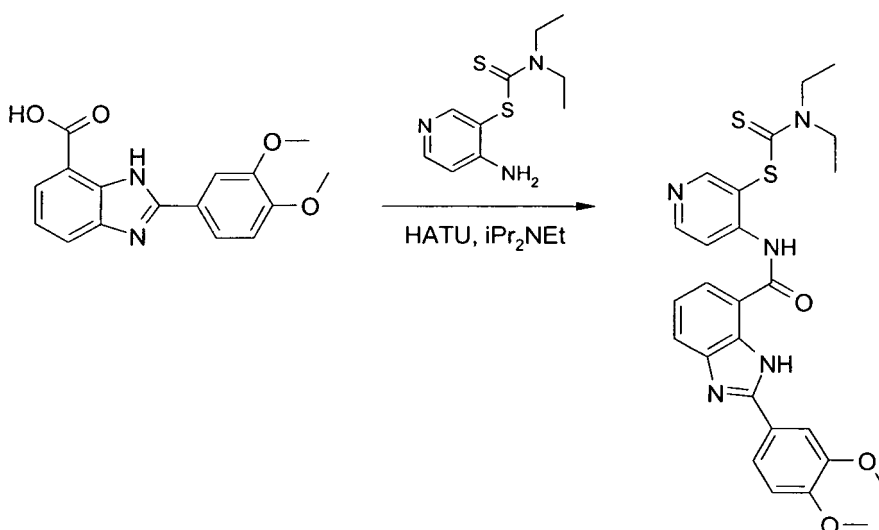
Preparation of 2-(3,4-dimethoxy-phenyl)-3H-benzimidazole-4-carboxylic acid:



- 5 2,3-Diaminobenzoic acid (0.25 g, 1.6 mmol) and 3,4-dimethoxybenzaldehyde (0.27 g, 1.6 mmol) were combined in DMF (6 mL). To this was added sodium metabisulfite (0.47 g, 2.5 mmol) and the reaction mixture was stirred with heating ($100^\circ\text{C} \times 20 \text{ h}$) in a sealed tube. Upon cooling, the mixture was partitioned between EtOAc and brine. The aqueous layer was extracted with EtOAc
- 10 (4 x 100 mL). The organic layer was dried (MgSO_4 and high vacuum) to afford 2-(3,4-dimethoxy-phenyl)-3H-benzimidazole-4-carboxylic acid. (Calc'd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_4$: 298.30, $[\text{M}+\text{H}]^+$ found: 299.1)

Preparation of diethyl-dithiocarbamic acid 4-{{2-(3,4-dimethoxy-phenyl)-3H-benzimidazole-4-carboxyl}-amino}-pyridin-3-yl ester:

15

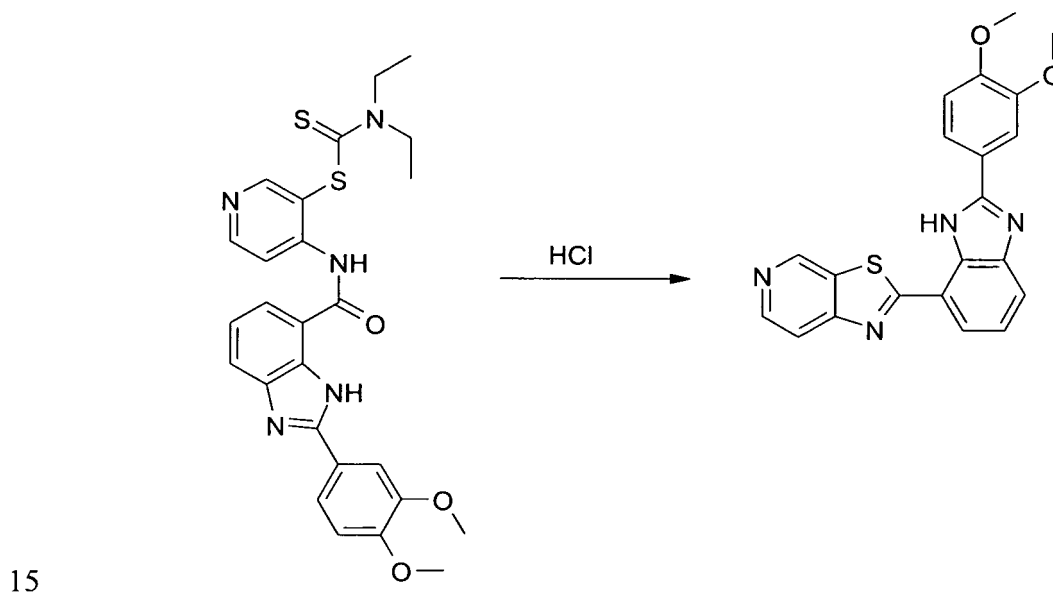


To a solution of 2-(3,4-dimethoxy-phenyl)-3H-benzimidazole-4-carboxylic acid (0.07 g, 0.2 mmol) in DMF (4 mL) was added HATU (0.13 g, 0.4 mmol), 4-

aminopyridin-3-yl diisopropylcarbamdithioate* (0.06 g, 0.2 mmol) and diisopropylethylamine (0.1 g, 0.7 mmol, 0.12 mL). The solution was stirred at RT (17 h). The reaction was partitioned between CH₂Cl₂ and brine. The organic layer was dried (MgSO₄) and concentrated. The crude material was purified on silica chromatography, 0-5% MeOH/CH₂Cl₂ to afford diethyl-dithiocarbamic acid 4-{{2-(3,4-dimethoxy-phenyl)-3H-benzoimidazole-4-carbonyl]-amino}-pyridin-3-yl ester. (Calc'd for C₂₆H₂₇N₅O₃S₂: 521/66. [M+H]⁺ found: 522.1)

*4-Aminopyridin-3-yl diisopropylcarbamdithioate was prepared according to the procedures outlined in Smith et al, *Sulfur Lett.* 1994 vol 17, p. 197 and E. Ma, *Molecules* 2003, vol 8, p. 678-686.

Preparation of 2-[2-(3,4-Dimethoxy-phenyl)-3H-benzimidazol-4-yl]-thiazolo[5,4-c]pyridine (Compound #3):



The diethyl-dithiocarbamic acid 4-{{2-(3,4-dimethoxy-phenyl)-3H-benzoimidazole-4-carbonyl]-amino}-pyridin-3-yl ester (0.1 g, 0.2 mmol) was suspended in a 2 N HCl solution (2 mL). The reaction was stirred with heating (100 °C x 30 min). Upon cooling, the solution was basified (1 N NaOH, pH 7.5). The resulting oil was solidified with the addition of EtOAc, and the solid was filtered, washed (MeOH)

and dried under high vac to afford 2-[2-(3,4-dimethoxy-phenyl)-3H-benzimidazol-4-yl]-thiazolo[5,4-c]pyridine as a yellow solid. (Calc'd for C₂₁H₁₆N₄O₂S: 388.4, [M+H]⁺ found: 389.1)

5 **EXAMPLE 3: SIRT1 Mass Spectrometry Assay**

The mass spectrometry based assay utilizes a peptide having 20 amino acid residues as follows: Ac-Glu-Glu-Lys(Biotin)-Gly-Gln-Ser-Thr-Ser-Ser-His-Ser-Lys(Ac)-Nle-Ser-Thr-Glu-Gly-Lys(5TMR)-Glu-Glu-NH₂ wherein K(Ac) is an acetylated lysine residue and Nle is a norleucine. The peptide is labelled with the
10 fluorophore 5TMR (excitation 540 nm/emission 580 nm) at the C-terminus. The sequence of the peptide substrate is based on p53 with several modifications.

The mass spectrometry assay was conducted as follows: 1/10 of K_m value for peptide substrate and 2/3 of the K_m value for βNAD⁺ were incubated with SIRT1 concentration giving less than 10% product conversion at 30 min reaction
15 time for a time course (0, 3, 6, 9, 12, 15, 20, 30 minutes) at 25°C in a reaction buffer (50 mM Tris-acetate pH 8, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 5 mM DTT, 0.05% BSA). Test compounds were added at 100 μM to the reaction or vehicle control, DMSO. After the incubation with SIRT1, 10% formic acid with 50 mM nicotinamide was added to stop the reaction. Determination of the mass of the
20 substrate peptide allows for precise determination of the degree of acetylation (i.e. starting material) as compared to deacetylated peptide (product).

The ability of structurally unrelated SIRT1 activators to activate SIRT1 at the same site was examined. Resveratrol and Compound #1 were chosen to determine if when combined, activate SIRT1 enzyme activity in an antagonistic, additive or
25 synergistic manner. The study was run using the cell-free SIRT1 Mass Spectrometry Assay in which resveratrol and Compound #1 were combined and tested in a concentration matrix. The concentrations of resveratrol tested were 300 μM, 100 μM, 33.3 μM, 11.1 μM, 3.7 μM, 1.23 μM, and 0.41 μM. The concentrations of Compound #1 tested in the matrix were 300 μM, 100 μM, 33.3
30 μM, 11.1 μM, 3.7 μM, 1.23 μM, 0.41 μM, 0.14 μM, 0.046 μM, and 0.015 μM. The Isobologram mass spectrometry assay was run as an end point assay as described

above. The resulting Isobologram (Figure 1) was used to evaluate the effect of the combination. For the analysis, a plot in Cartesian coordinates of a dose combination that produce the same effect level is the basis for an Isobologram. If two compounds have variable potency, a constant relative potency (R) - which is the amount of compound needed to achieve the same fold activity (e.g. EC1.25 in this study) - is selected for the X and Y intercepts for Isobologram analysis. The X and Y axes in Figure 1 represent the doses of resveratrol and Compound #1. The concentration of both compounds which corresponds to the respective EC1.25 value is used as an intercept on both the X and Y axes. Using these two intercepts, a hypothetical line (Figure 1 dotted line) called the line of additivity is drawn between the two points. Experimental data obtained by the logarithmic titration of the two compounds mixed as a dose pair in a matrix a, which yield the same effect level (EC1.25), is plotted on the Isobologram. Statistical comparison of the line of additivity and the curve arising from experimental two drug dose combinations indicates if an effect is additive. Points falling below and above the line of additivity are subjected to regression analysis. Experimental data that is higher than the line of additivity is interpreted antagonistic and experimental data that is lower is interpreted as synergistic, and experimental that fall on the line of additivity is considered additive.

Results demonstrate that resveratrol and Compound #1, when combined, increase SIRT1 enzyme activity in the SIRT1 Mass Spectrometry Assay in an additive manner. The data suggest that resveratrol and Compound #1 may activate SIRT1 by binding to the same site on the enzyme.

EXAMPLE4 : SIRT1 Truncation Series

To begin to define the site to which the small molecule activators bind, a large series of SIRT1 truncation mutants were generated (Figure 2). The deletion series was expressed in *E. coli* as described in Example 1. Figure 2 shows information on the construct, including starting and ending amino acid positions, resulting size, the affinity tag used for purification as well as the protease site for removal of the tag. In addition, expression level as determined in *E. coli*, acetylation activity and activatability for most of the constructs is also shown.

Expression levels of the SIRT1 variants in *E. coli* are quantified as 'low' for less than 5 mg/L of purified SIRT1 variant/starting L of culture, 'medium' for 5-15 mg/L, and 'high' for greater than 15 mg/L. The full-length construct of SIRT1 expresses poorly in *E. coli*. Construct SIRT1-D4 demonstrates that deletion of a
5 portion of the N-terminus permits higher levels of expression.

Deacetylation activity is measured for the deletion mutants using the mass spec assay. Deacetylation activity is quantified as 'high' for 100% activity when compared to the activity of full-length SIRT1, 'medium' when the activity is at least 25%, and 'low' when the activity of the deletion mutants is less than 5% of the full-
10 length SIRT1 deacetylation activity.

The ability of the deletion mutants to be activated by sirtuin modulating compounds was measured in the mass spec assay. Resveratrol, Compound #2 and Compound #3 were used in the assays. Activation is quantified as 'high' when a compound results in a two-fold activation or greater and 'low' when the activation is
15 less than a 1.5-fold increase.

Figure 3 depicts graphically a series of the SIRT1 variants. Enzyme activity refers to deacetylation activity of the polypeptides in the presence of DMSO alone (DMSO) (e.g., in the absence of a sirtuin modulating compound) as well as the deacetylase activity of the polypeptides in the presence of a
20 sirtuin modulating compound (Comp. #2). The SIRT1-E5c construct retains specific activity and activation properties essentially identical to full length SIRT1 but is expressed at high levels in *E. coli*. SIRT1-A8 (amino acid residues 150-670 of SEQ ID NO: 1) and B8 (amino acid residues 170-670 of SEQ ID NO: 1) retain similar activity and activation as the E5c construct, while SIRT1-C8 (amino acid residues
25 190-670 of SEQ ID NO: 1) and D8 (amino acid residues 210-670 of SEQ ID NO: 1) exhibit a marked decrease in enzyme activity. The latter two fragments express poorly in *E. coli* and may lose activity simply due to misfolding. The SIRT1-E8 (amino acid residues 225-670 of SEQ ID NO: 1) construct exhibits enzymatic activity comparable to E5c, but loses the ability to be activated by Compound #2
30 suggesting that the stretch of amino acids 183-225 is important in defining the binding site for small molecule activators of the Compound #1 class of compounds.

The SIRT1 deletion experiments indicate that the region of amino acids 183-225 of SEQ ID NO: 1 is critical for maintaining activation of SIRT1 by these compounds and is involved in defining the allosteric binding site. It is possible that acetylated peptide substrate binding to SIRT1 induces a conformational change that exposes an allosteric site in this region of the enzyme. An endogenous regulator of SIRT1 has yet to be identified and it is tempting to speculate that an endogenous activator of SIRT1 exists and may be increased following calorie restriction and other mild physiological stresses.

10 EQUIVALENTS

The present invention provides among other things sirtuin variants and methods of use thereof. While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

INCORPORATION BY REFERENCE

20 All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

25 Also incorporated by reference in their entirety are any polynucleotide and polypeptide sequences which reference an accession number correlating to an entry in a public database, such as those maintained by The Institute for Genomic Research (TIGR) (www.tigr.org) and/or the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

30

CLAIMS:

1. A SIRT1 variant, wherein the variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of the corresponding wild type SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound.
2. The SIRT1 variant of claim 1, wherein the variant comprises amino acid residues 183-664, 141-747, 183-664, 183-705, 183-724, 155-664, 155-747, 164-664, 164-747, 172-664, 219-664, 150-670, or 170-670 of SEQ ID NO:1.
3. The SIRT1 variant of claim 1, wherein the variant is selected from the group consisting of: (i) a fragment of SEQ ID NO: 1 having an N-terminus that falls between amino acid residues 154 to 184 of SEQ ID NO: 1 and a C-terminus that falls between amino acid residues 663-748 of SEQ ID NO:1, (ii) a polypeptide which is at least 90% identical to the fragment of (i), or (iii) the fragment of (i) with 1 to about 10 conserved amino acid changes.
4. A fusion protein comprising the SIRT1 variant of claim 1 and a polypeptide that increases solubility or facilitates purification, identification, detection, structural characterization, or cellular uptake of the SIRT1 variant.
5. The SIRT1 variant of claim 1 which is a human SIRT1 variant.
6. A nucleic acid that encodes a SIRT1 variant of any of claims 1-5.
7. The nucleic acid of claim 6, wherein the nucleic acid is selected from the group consisting of: (i) a nucleic acid which is a fragment of SEQ ID NO: 2 having a 5' end that falls between nucleotide residues 460-550 of SEQ ID NO: 2 and a 3' end that falls nucleotide residues 1987-2242 of SEQ ID NO: 2; (ii) a nucleic acid comprising nucleotide residues 547-1992, 421-2241, 547-2115, 547-2172, 463-

1992, 463-2241, 490-1992, 490-2241, 514-1992, 655-1992, 448-2010, or 508-2010 of SEQ ID NO: 2, (iii) a nucleic acid that is at least 90% identical to the nucleic acid of (i) or (ii); or (iv) a nucleic acid that hybridizes under stringent conditions to the nucleic acid of (i) or (ii).

8. An expression vector comprising a nucleic acid of any one of claims 6-7.

9. A host cell comprising a nucleic acid of any one of claims 6-7.

10. A transgenic non-human mammal, a majority of whose cells harbor a transgene comprising a nucleic acid sequence described in claim 6.

11. The transgenic non-human mammal of claim 10 wherein SIRT1 deacetylation activity is increased in a majority of cells.

12. The transgenic non-human mammal of claim 10 wherein the transgene further comprises a control sequence operably linked to the sequence.

13. The transgenic non-human mammal of claim 12, wherein the control sequence is a promoter.

14. The transgenic non-human mammal described in claim 10 wherein the life span of the mammal is increased with respect to a nontransgenic mammal of the same species.

15. The transgenic non-human mammal of claim 10, wherein the transgene is incorporated into the genome.

16. A method for identifying a compound that modulates SIRT1 activity, comprising:

(a) contacting a peptide substrate pool with a SIRT1 variant in the presence of a compound, wherein members of said peptide substrate pool comprise at least one

acetylated amino acid side chain and wherein the SIRT1 variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound, and (b) determining the level of acetylation of the peptide substrate pool, wherein a change in the level of acetylation of the peptide substrate pool in the presence of the test compound as compared to a control is indicative of a compound that modulates SIRT1.

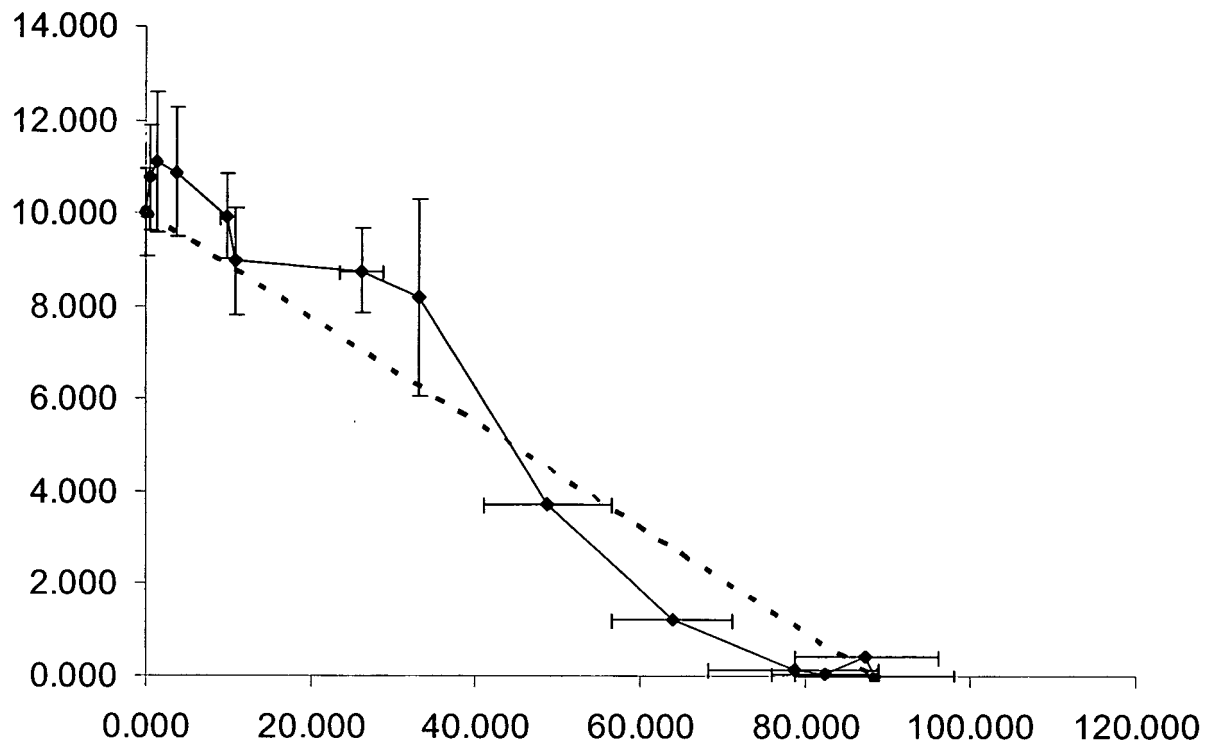
17. The method of claim 16, further comprising contacting a cell with the compound and determining if the compound increases the life span of the cell.

18. A method for determining SIRT1 activity, comprising: (a) contacting a peptide substrate pool with a SIRT1 variant, wherein members of said peptide substrate pool comprise at least one acetylated amino acid side chain, and wherein the SIRT1 variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of full length SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound, and (b) determining if the acetylated amino acid side chain in the peptide substrate pool is deacetylated.

19. A method of deacetylating at least one amino acid residue in a polypeptide comprising combining a polypeptide having at least one acetylated amino acid with a SIRT1 variant, wherein the SIRT1 variant (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of the corresponding wild type SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound.

20. The method of claim 19, wherein NAD or an NAD-like compound is added to the mixture.

21. A method of extending the life span of a eukaryotic cell comprising introducing into the cell a nucleic acid of claim 6.
22. The method of claim 21, wherein life span is determined by measuring the population doubling of the cell.
23. A method of treating a subject suffering from a disease or disorder that would benefit from increased sirtuin activity, comprising administering to the subject an effective amount of a SIRT1 variant of claim 1 or a nucleic acid of claim 6.
24. The method of claim 23, comprising administering to the subject an expression vector comprising a nucleic acid that encodes the SIRT1 variant.
25. The method of claim 23, wherein the disease or disorder is one or more of the following: aging, stress, cardiovascular disease, cancer, obesity, inflammatory disease, mitochondrial-associated diseases, neuronal disorders, blood coagulation disorders, diabetes, flushing, and ocular disorders.
26. A vertebrate cell comprising a nucleic acid of claim 6.
27. A method for mimicking the effects of calorie restriction in a eukaryotic cell, comprising introducing into the eukaryotic cell a nucleic acid of claim 6.
28. A method for mimicking the effects of calorie restriction in a eukaryotic cell, comprising contacting the cell with a SIRT1 variant that (i) may be expressed in *E. coli* at a concentration of at least 5 mg/L, (ii) has deacetylase activity that is substantially equivalent to the deacetylase activity of the corresponding wild type SIRT1, and (iii) the deacetylase activity may be activated by at least 2-fold in the presence of a sirtuin activating compound

**FIGURE 1**

Name	Start AA	End AA	Size (kDa)	Tag	Protease site	Expression level ¹	Acetylation activity ²	Compound Activation ³
SIRT1 FL	1	747	83	N-term His		Low	High	High
pGSTSIRT1 A1	2	580	89	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 A2	2	598	90	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 A3	2	634	95	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 A4	2	747	108	N-term GST	TEV	Low	High	High
pGSTSIRT1 B1	53	580	85	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 B2	53	598	84	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 B3	53	634	90	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 B4	53	747	103	N-term GST	TEV	Low	High	High
pGSTSIRT1 C1	116	580	78	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 C2	116	598	79	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 C3	116	634	84	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 C4	116	747	97	N-term GST	TEV	Low	High	High
pGSTSIRT1 D1	141	580	75	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 D2	141	598	76	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 D3	141	634	81	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 D4	141	747	94	N-term GST	TEV	Low	High	High
pGSTSIRT1 E1	183	580	70	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 E2	183	598	71	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 E3	183	634	76	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 E4	183	747	89	N-term GST	TEV	Low	High	High
pGSTSIRT1 F1	214	580	67	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 F2	214	598	68	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 F3	214	634	73	N-term GST	TEV	Low	Low	Low
pGSTSIRT1 F4	214	747	86	N-term GST	TEV	Low	Low	Low
SIRT1 192	192	747	61	N-term His	TEV	Low	N/A	N/A
pHisSIRT1 A1	2	580	67	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 A2	2	598	64	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 A3	2	634	69	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 A4	2	747	82	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 B1	53	580	59	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 B2	53	598	58	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 B3	53	634	64	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 B4	53	747	77	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 C1	116	580	52	N-term His	TEV	N/A	N/A	N/A

FIGURE 2

Name	Start AA	End AA	Size (kDa)	Tag	Protease site	Expression level ¹	Acetylation activity ²	Compound Activation ³
pHisSIRT1 C2	116	598	53	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 C3	116	634	22	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 C4	116	747	71	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 D1	141	580	49	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 D2	141	598	50	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 D3	141	634	55	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 D4	141	747	68	N-term His	TEV	Medium	High	High
pHisSIRT1 E1	183	580	44	N-term His	TEV	Low	N/A	N/A
pHisSIRT1 E2	183	598	45	N-term His	TEV	Low	N/A	N/A
pHisSIRT1 E3	183	634	50	N-term His	TEV	Low	N/A	N/A
pHisSIRT1 E4	183	747	63	N-term His	TEV	Low	High	High
SIRT1 E4C	183	747	64	C-term His	PPS	Low	High	High
pHisSIRT1 F1	214	580	41	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 F2	214	598	42	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 F3	214	634	47	N-term His	TEV	N/A	N/A	N/A
pHisSIRT1 F4	214	747	60	N-term His	TEV	N/A	N/A	N/A
SIRT1 E5C	183	664	56	C-term His	PPS	Medium	High	High activated by Comp. #2, #3, and Resveratrol
SIRT1 E6C	183	705	60	C-term His	PPS	Medium	High	High
SIRT1 E7C	183	724	62	C-term His	PPS	Low	High	High
SIRT1 E8C	183	736	63	C-term His	PPS	Low	High	High
SIRT1 E5N	183	664	56	N-term His	TEV	Low	Low	Low
SIRT1 E6N	183	705	60	N-term His	TEV	Medium	High	High
SIRT1 E7N	183	724	62	N-term His	TEV	Medium	N/A	N/A
SIRT1 E8N	183	736	63	N-term His	TEV	N/A	N/A	N/A
SIRT1 E9C	183	648	52	C-term His	PPS	Low	N/A	N/A
SIRT1 E10C	183	638	51	C-term His	PPS	Low	N/A	N/A
SIRT1 G5N	155	664	57	N-term His	TEV	Medium	Low	Low
SIRT1 G4N	155	747	66	N-term His	TEV	Medium	High	High
SIRT1 H5N	164	664	56	N-term His	TEV	Medium	High	High
SIRT1 H4N	164	747	65	N-term His	TEV	Medium	High	High
SIRT1 I5N	172	664	55	N-term His	TEV	Low	High	High
SIRT1 I4N	172	747	64	N-term His	TEV	Low	Low	Low
SIRT1-G5C	156	664	57	C-term His	PPS	Medium	High	N/A
SIRT1-H5C	163	664	56	C-term His	PPS	Medium	High	N/A

FIGURE 2 Con't

Name	Start AA	End AA	Size (kDa)	Tag	Protease site	Expression level ¹	Acetylation activity ²	Compound Activation ³
SIRT1-I5C	172	664	55	C-term His	PPS	Medium	Low	N/A
SIRT1-J5C	219	664		C-term His	PPS	Medium	Low	N/A
SIRT1-K5C	225	664		C-term His	PPS	Medium	High	Low- activated by Comp. #3
SIRT1-L5C	230	664		C-term His	PPS	High	High	N/A
SIRT1-M5C	235	664		C-term His	PPS	High	High	N/A
SIRT1-N5C	240	664		C-term His	PPS	High	High	N/A
SIRT1 E4-K233A, K235A, K236A, K238A	183	747	63	N-term His	TEV	Low	N/A	N/A
SIRT1 E4 synthetic	183	747	63	N-term His	TEV	Low	N/A	N/A
SIRT1 192 synthetic	192	747	61	N-term His	TEV	Low	N/A	N/A
SIRT1 FL synthetic	1	747	83	N-term His	TEV	Low	N/A	N/A
SA4/8	150	670	59	N and C- term His	N term Thrombine C term none	Medium	High	High- activated by Comp. #2 and #3
SB8	170	670	56	C-term His	none	Low	High	High- activated by Comp. #2 and #3
SC4/8	190	670	54	N and C- term His	N term Thrombine C term none	Low	Low	Low- activated by Comp. #2 and #3
SD4/8	210	670	52	N and C- term His	N term Thrombine C term none	Low	Low	High- activated by Comp. #2 and #3
SE4/8	225	670	50	N and C- term His	N term Thrombine C term none	High	High	Low- activated by Comp. #2 and #3
SF4/8	240	670	48	N and C- term His	N term Thrombine C term none	High	High	Low- activated by Comp. #3
SB4	170	576	46	N-term His	Thrombine	Low	Low	High- activated by Comp. #3

FIGURE 2 Con't

Name	Start AA	End AA	Size (kDa)	Tag	Protease site	Expression level ¹	Acetylation activity ²	Compound Activation ³
SIRT1 E5C-K203A	183	664	56	C-term His	PPS	Medium	High	High- activated by Comp. #3
SIRT1 E5C-E512A, K513A	183	664	56	C-term His	PPS	Medium	High	High- activated by Comp. #3
SIRT1 E5C-E577A, K578A, Q580A	183	664	56	C-term His	PPS	Medium	High	High- activated by Comp. #3
SIRT1 E5C-E593A, Q594A, E596A	183	664	56	C-term His	PPS	Medium	High	High- activated by Comp. #3
SIRT1 E5C-E230K	183	664	56	C-term His	PPS	Medium	High	N/A
SIRT1 E4N-K203A	183	747	63	N-term His	TEV	N/A	N/A	N/A
SIRT1 E4N-E512A, K513A	183	747	63	N-term His	TEV	N/A	N/A	N/A
SIRT1 E4N-E577A, K578A, Q580A	183	747	63	N-term His	TEV	N/A	N/A	N/A
SIRT1 E4N-E593A, Q594A, E596A	183	747	63	N-term His	TEV	N/A	N/A	N/A

Expression Levels¹

High: >15 mg/L culture purified
Medium: 5-15 mg/L culture purified
Low: <5 mg/L culture purified

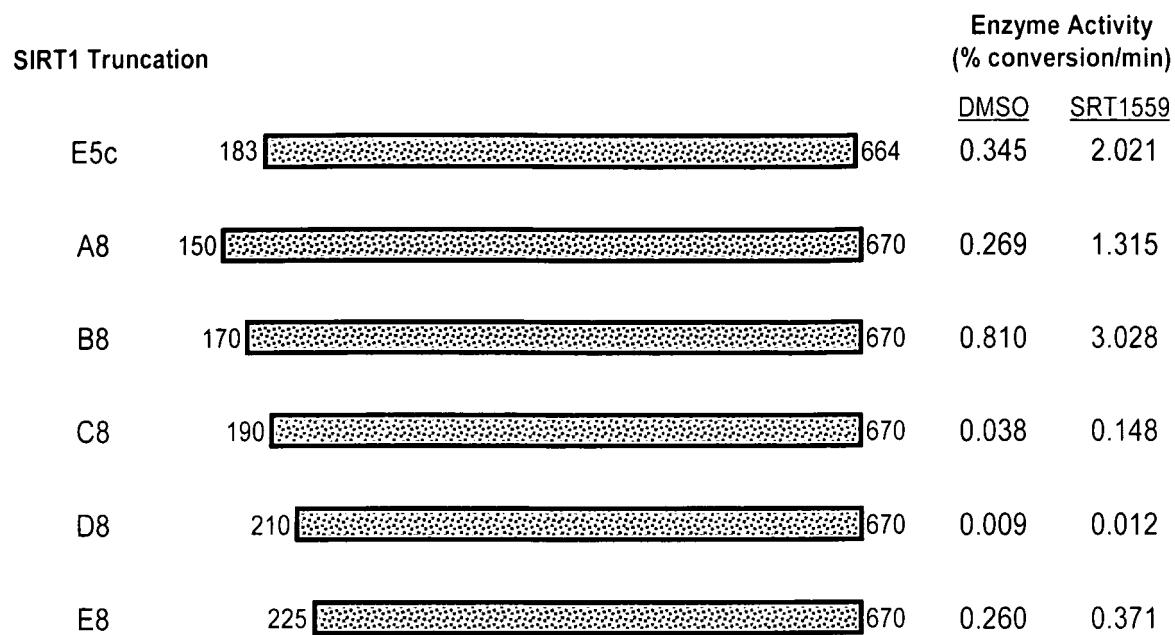
Acetylation Activity²

High: 100% full length activity
Medium: 25% full length activity
Low: <5% full length activity

Activation³

High: >2 fold activation
Low: <1.5 fold activation

FIGURE 2 Con't

**FIGURE 3**

1 M A D E A A L A L Q P G G S P S A A G A
1 ATGGCGGACGAGGCGGCCCTCGCCCTTCAGCCCGGCGGCTCCCCCTCGGCGGCGGGGGCC

21 D R E A A S S P A G E P L R K R P R R D
61 GACAGGGAGGCCGCGTCGTCCCCCGCGGGGAGCCGCTCCGCAAGAGGCCGCGGAGAGAT

41 G P G L E R S P G E P G G A A P E R E V
121 GGTCCCGGCCCTCGAGCGGAGCCCGGGCGAGCCCGGTGGGGCGGCCCCAGAGCGTGAGGTG

61 P A A A R G C P G A A A A A L W R E A E
181 CCGGCGGCGGCCAGGGGCTGCCCGGTGCGGCGGCGGCGGCGCTGTGGCGGGAGGCGGAG

81 A E A A A A G G E Q E A Q A T A A A G E
241 GCAGAGGCGGCGGCGGCAGGCGGGGAGCAAGAGGCCCGAGGCGACTGCGGCGGCTGGGGAA

101 G D N G P G L Q G P S R E P P L A D N L
301 GGAGACAATGGGCCGGGCCTGCAGGGCCCATCTCGGGAGCCACCGCTGGCCGACAACCTTG

121 Y D E D D D D E G E E E E E A A A A A I
361 TACGACGAAGACGACGACGACGAGGGCGAGGAGGAGGAAGAGGCGGCGGCGGCGGCGATT

141 G Y R D N L L F G D E I I T N G F H S C
421 GGGTACCGAGATAACCTTCTGTTCGGTGATGAAATTATCACTAATGGTTTTTCATTCCTGT

161 E S D E E D R A S H A S S S D W T P R P
481 GAAAGTGATGAGGAGGATAGAGCCTCACATGCAAGCTCTAGTGACTGGACTCCAAGGCCA

181 R I G P Y T F V Q Q H L M I G T D P R T
541 CGGATAGGTCCATATACTTTTGTTCAGCAACATCTTATGATTGGCACAGATCCTCGAACA

201 I L K D L L P E T I P P P E L D D M T L
601 ATTCTTAAAGATTTATTGCCGGAACAATACCTCCACCTGAGTTGGATGATATGACACTG

221 W Q I V I N I L S E P P K R K K R K D I
661 TGGCAGATTGTTATTAATATCCTTTTCAGAACCACCAAAAAGGAAAAAAGAAAAGATATT

241 N T I E D A V K L L Q E C K K I I V L T
721 AATACAATTGAAGATGCTGTGAAATTACTGCAAGAGTGCAAAAAAATTATAGTTCTAACT

261 G A G V S V S C G I P D F R S R D G I Y
781 GGAGCTGGGGTGCTGTGTTTCATGTGGAATACCTGACTTCAGGTCAAGGGATGGTATTTAT

281 A R L A V D F P D L P D P Q A M F D I E
841 GCTCGCCTTGCTGTAGACTTCCAGATCTTCCAGATCCTCAAGCGATGTTTGATATTGAA

301 Y F R K D P R P F F K F A K E I Y P G Q
901 TATTTTCAGAAAAGATCCAAGACCATTCTTCAAGTTTGCAAAGGAAATATATCCTGGACAA

321 F Q P S L C H K F I A L S D K E G K L L
961 TTCCAGCCATCTCTCTGTCAAAATTCATAGCCTTGTCAGATAAGGAAGGAAAACCTACTT

341 R N Y T Q N I D T L E Q V A G I Q R I I
1021 CGCAACTATACCCAGAACATAGACACGCTGGAACAGGTTGCGGGAATCCAAAGGATAATT

361 Q C H G S F A T A S C L I C K Y K V D C
1081 CAGTGTTCATGGTTTCCTTTGCAACAGCATCTTGCCTGATTTGTAAATACAAAGTTGACTGT

381 E A V R G D I F N Q V V P R C P R C P A
1141 GAAGCTGTACGAGGAGATATTTTAAATCAGGTAGTTCCTCGATGTCCTAGGTGCCAGCT

401 D E P L A I M K P E I V F F G E N L P E
1201 GATGAACCGCTTGCTATCATGAAACCAGAGATTGTGTTTTTTGGTGAAAATTTACCAGAA

421 Q F H R A M K Y D K D E V D L L I V I G
1261 CAGTTTCATAGAGCCATGAAGTATGACAAAGATGAAGTTGACCTCCTCATTGTTATTGGG

441 S S L K V R P V A L I P S S I P H E V P
1321 TCTTCCCTCAAAGTAAGACCAGTAGCACTAATTCCAAGTTCATACCCCATGAAGTGCCT

461 Q I L I N R E P L P H L H F D V E L L G
1381 CAGATATTAATTAATAGAGAACCTTTGCCTCATCTGCATTTTGATGTAGAGCTTCTTGGG

481 D C D V I I N E L C H R L G G E Y A K L
1441 GACTGTGATGTCATAATTAATGAATTGTGTCATAGGTTAGGTGGTGAATATGCCAACTT

501 C C N P V K L S E I T E K P P R T Q K E
1501 TGCTGTAACCCTGTAAAGCTTTCAGAAATTACTGAAAAACCTCCACGAACACAAAAAGAA

521 L A Y L S E L P P T P L H V S E D S S S
1561 TTGGCTTATTTGTCAGAGTTGCCACCCACACCTCTTCATGTTTCAGAAGACTCAAGTTCA

541 P E R T S P P D S S V I V T L L D Q A A
1621 CCAGAAAGAACTTCACCACCAGATTCTTCAGTGATTGTCACACTTTTAGACCAAGCAGCT

561 K S N D D L D V S E S K G C M E E K P Q
1681 AAGAGTAAATGATGATTTAGATGTGTCTGAATCAAAGGTTGTATGGAAGAAAAACCACAG

581 E V Q T S R N V E S I A E Q M E N P D L
1741 GAAGTACAACTTCTAGGAATGTTGAAAGTATTGCTGAACAGATGGAAAATCCGATTTG

601 K N V G S S T G E K N E R T S V A G T V
1801 AAGAATGTTGGTTCTAGTACTGGGGAGAAAAATGAAAGAACTTCAGTGGCTGGAACAGTG

621 R K C W P N R V A K E Q I S R R L D G N
1861 AGAAAATGCTGGCCTAATAGAGTGGCAAAGGAGCAGATTAGTAGGCGGCTTGATGGTAAT

641 Q Y L F L P P N R Y I F H G A E V Y S D
1921 CAGTATCTGTTTTTGCCACCAAATCGTTACATTTTCCATGGCGCTGAGGTATATTACAGAC

661 S E D D V L S S S S C G S N S D S G T C
1981 TCTGAAGATGACGTCTTATCCTCTAGTTCTTGTGGCAGTAACAGTGATAGTGGGACATGC

681 Q S P S L E E P M E D E S E I E E F Y N
2041 CAGAGTCCAAGTTTAGAAGAACCCATGGAGGATGAAAGTGAATTGAAGAATTCTACAAT

701 G L E D E P D V P E R A G G A G F G T D
2101 GGCTTAGAAGATGAGCCTGATGTTCCAGAGAGAGCTGGAGGAGCTGGATTTGGGACTGAT

721 G D D Q E A I N E A I S V K Q E V T D M
2161 GGAGATGATCAAGAGGCAATTAATGAAGCTATATCTGTGAAACAGGAAGTAACAGACATG

741 N Y P S N K S
2221 AACTATCCATCAAACAAATCA

```

1 -----MTIPHKYAVSKTSENKVSNTVSPTQDKDAIRKQDDIINN-DEPSHKKIKVAQ yeast SIR2
1 MADEVALALQAAGSPSAA-AAMEAASQPADEPLRKRPRRDGPGLGRSPGEPSSAAVAP--A mouse SIRT1
1 MADEAALALQPGGSPSAAGADREAASSPAGEPLRKRPRRDGPGLERSPGEPGGAAPEREV human SIRT1
1 ----- human SIRT2
1 -----MAFWGWRAAAAL-----RLWGRVVER--VEAGGGVGP--- human SIRT3
1 ----- human SIRT4
1 ----- human SIRT5
1 ----- human SIRT6
1 ----- human SIRT7

54 PDSLRETNTTDPLGHTKAALGEVASMELKPTNDMDPLAVSAASVVMSNDVLKPETPKGP yeast SIR2
58 AAGCEAASAAAPALWREAAGAAASA--EREAPATAVAGDNGSGLR---REPRAADDF mouse SIRT1
61 PAAARGCPGAAAAALWREAEEAAAAGGEQEAQATAAAGEGDNPGPLQGSPREPPLADNL human SIRT1
1 -----MAEP-DPSHLE---T-QAG-KVQ human SIRT2
31 -----FQACGCRLVLGGRDDVSAG---LRGSHGARGEPLDPAARPLQ---R-PPRPEVP human SIRT3
1 -----MKMS-----FA---LTFRSAKGR human SIRT4
1 -----MRPLQ---IVPSRLISQ human SIRT5
1 -----MSVN-----YAAGLS human SIRT6
1 -----MAAGGLSRSERKAA---ERVRLREEQQRERLRQVS---RILRKAAAE human SIRT7

114 IISKNPNGIFYGPSFTKRESLNARMFLKYGAHKFLDLYLPEDLNSLYIYYLIKLLGF yeast SIR2
113 DDDEGEED-----EAAAAAAAAAIGYRDNLLLTGGLTNGFHSCESDDDDRSTSHA mouse SIRT1
121 YDEDDDDDEG-----EEEEEEAAGYRDNLLFGDEIITNGFHSCESEEDDRASHA human SIRT1
19 EAQSDSDS-----EG-----GAAGGEADMDFLRNLFS-----QTLSLG human SIRT2
77 RAFRRQPR-----AAPSFFSSIKGGRSISFSVGASS-----VVGSGG human SIRT3
16 WIANPSQ-----P---CS-----KASIG--LFV-----PASPP human SIRT4
15 LYCGLKPPA-----STRNQICLKMARPS-----S human SIRT5
11 PYADKG-----KCGLP---E-----IFDPP human SIRT6
43 RSAEEGRLLA-----ESADLVTELQGRSRRREGLKRRQEE-----VCDDP human SIRT7

174 EVKDQALIGTIN-----SIVHINSQERVQDLGSAISVTNVEDPLAKKQTVRLIKDLQ yeast SIR2
164 SSSDWTPRPRIGPYTFVQQHLMIGTDPRTILKDLLPETIPPELDDMTLWQIVINILSEP mouse SIRT1
172 SSSDWTPRPRIGPYTFVQQHLMIGTDPRTILKDLLPETIPPELDDMTLWQIVINILSEP human SIRT1
53 SQKERL-----LDELTLEGVARYMQSE- human SIRT2
117 SSDK-----GKLSLQDVAELIRAR- human SIRT3
39 LDPE-----KVKELRQRFITLS- human SIRT4
39 SMAD-----FRKFFAK- human SIRT5
28 EELER-----KVWELARLVWQS- human SIRT6
83 EELRG-----KVRELASAVRNA- human SIRT7

226 RAINKVLCTRLRLSNFFTIDHFIQKLHTARKILVLTGAGVSTSLGIPDFRS-SEGIFYSKI yeast SIR2
224 -----PKRKKRKDINTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRS-RDGIYARL mouse SIRT1
232 -----PKRKKRKDINTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRS-RDGIYARL human SIRT1
75 -----R-----CRRVICLVGAGISTSAGIPDFRSPSTGLYDNL human SIRT2
136 -----A-----CQRVVVMVGAGISTPSGIPDFRSPGSGLYSNL human SIRT3
55 -----KRLVMTGAGISTESGIPDYRSEKVGLYART human SIRT4
50 -----AKHIVIIISGAGVSAESGVPTFRG-AGGYWRKW human SIRT5
45 -----SSVVFHTGAGISTASGIPDFRGP-HGVVTME human SIRT6
100 -----KYLVVYTGAGISTAASIPDYRGP-NGVWTL human SIRT7

285 KH--LGLDDPDQVFNYNIFMHDPSVFYNIANMVLPEEKIYSPHLSFIKMLQ---MKGKL yeast SIR2
276 AVDFPDLDPQAMFDIEYFRKDRPFFKFAKEIYPGQFQPSLCHKFIALSD---KEGKL mouse SIRT1
284 AVDFPDLDPQAMFDIEYFRKDRPFFKFAKEIYPGQFQPSLCHKFIALSD---KEGKL human SIRT1
108 EK--YHLPYPEAIFEISYFKKHPEFFALAKELYPGQFKPTICHYFMRLK---DKGLL human SIRT2
169 QQ--YDLPYPEAIFELPFFFHNPFPFTLAKELYPGNYKPNVTHYFLRLH---DKGLL human SIRT3
86 DRR--PIQHGFVRSAPIRQRYWARNFVGPWFSSHQ--PNPAHWALSTWE---KLGLK human SIRT4
81 QA--QDLATP---LAFAHNPSRVWFEFYHYRREVMGSK-EPNAGHRAIAECETRLGKQGR human SIRT5
75 ER-----GLAPKFDTTFESAR--PTQTHMALVQLE---RVGLL human SIRT6
130 QK-----GRS-VSAADLSEAE--PTLTHMSITRLH---EQKLV human SIRT7

```

339 LRNYTQNIIDNLESYAGISTDKLVQCHGSFATATCV---TCHWNLP---GERIFNK----- yeast SIR2
 332 LRNYTQNIIDTLEQVAGIQR--ILQCHGSFATASCL---ICKYKVD---CEAVRGD----- mouse SIRT1
 340 LRNYTQNIIDTLEQVAGIQR--IIQCHGSFATASCL---ICKYKVD---CEAVRGD----- human SIRT1
 162 LRCYTQNIIDTLERIALGLEQEDLVEAHGTFYTSCHCVSA-SCRHEYP---LSWMKEK----- human SIRT2
 223 LRLYTQNIIDGLERVSGIPASKLVEAHGTFASATCT---VCQRFPF---GEDIRAD----- human SIRT3
 138 YWLVTQNVDAHLTKAGSRR--LTELHGCMRVLCL---DCGEQTP---RGVLQER----- human SIRT4
 135 VVVITQNIIDELHRKAGTKN--LLEIHGSLFKTRCTSCGVVAENYKSPICPALSGKGAPPEP human SIRT5
 108 RFLVSQNVQDGLHVRSGFPRDKLAELHGNMFVEECA---KCKTQYV---RDTVVG----- human SIRT6
 162 QHVVSQNCQDGLHRLSGLPRTAISELHGNMYIEVCT---SCVPN-----REYVR----- human SIRT7

 388 -----IRNLELPLCPYCYKKRREYFPEGYNKVGVAASQGSMSERPPYILNSYGVLPKD yeast SIR2
 379 -----IFNQVPRCPRCPAD-----EPLAIMKPE mouse SIRT1
 387 -----IFNQVPRCPRCPAD-----EPLAIMKPE human SIRT1
 213 -----IFSEVTPKCEDCQS-----LVKPD human SIRT2
 272 -----VMADRVPRCPVCTG-----VVKPD human SIRT3
 185 --FQVLNPTWSAEAHGLAPDGD---V-----FLSEEQVRSFQVPTCVQCGHLKPD human SIRT4
 193 GTQDASIPVEKLPREEAGCG-----GLLRPH human SIRT5
 156 ----TMGLKATGRCLTVAKAR-----GLRACRGELRDT human SIRT6
 207 ----VFDVTERTALHRHQTGR-----TCHKCGTQLRDT human SIRT7

 442 ITFFGE--ALPN-KFHKSIREIDILECDLLICIGTSLKVAPVS---EIVNMVPSH-VPQVL yeast SIR2
 403 IVFFGE--NLPE-QFHRAKMYDKDEVLLIVIGSSSLKVRPVA---LIPSSIPHE-VPQIL mouse SIRT1
 411 IVFFGE--NLPE-QFHRAKMYDKDEVLLIVIGSSSLKVRPVA---LIPSSIPHE-VPQIL human SIRT1
 232 IVFFGE--SLPA-RFFSCMQSDFLKVDLLVMGTSLQVQPFA---SLISKAPLS-TPRLL human SIRT2
 291 IVFFGE--PLPQ-RFLHVV-DFPMADLLIILGTSLEVEPFA---SLTEAVRSS-VPRL human SIRT3
 231 VVFFGD--TVNP-DKVDVHKRVKEADSLVVGSSSLQVYSGY-RFILTAW--EKKLPIAI human SIRT4
 220 VVWFGE--NLDP-AILEEVDRELAHCDLCLVVGTSVVYPAA---MFAPQVAARGVPVAE human SIRT5
 185 ILDWED--SLPD-RDLALADEASRNADLSITLGTSLQIRPSG-NLPLATK--RRGRLVI human SIRT6
 236 IVHFERGTLGQPLNWEATEAASRADTILCLGSSSLKVLKYPRLWCMTKPPSRRPKLYI human SIRT7

 495 INRD-----PVKHAEFDLISL yeast SIR2
 456 INREPLPHLHFDVELLGDCDVIINELCHRLGGEYAKLCCNPVKLSEITEKPPRPQKELVH mouse SIRT1
 464 INREPLPHLHFDVELLGDCDVIINELCHRLGGEYAKLCCNPVKLSEITEKPPRTQKELAY human SIRT1
 285 INKE-----KAGQSDPFL-GMIMGLG-----GGMDFDSKKAYRDVAW human SIRT2
 343 INRD-----LVGP-----LAWHPR--SRDVAQ human SIRT3
 285 LNIGP-----TRSDDLACLKL human SIRT4
 274 FNTET-----TPATNRFRFHF human SIRT5
 239 VNLQP-----TKHDRHADLRI human SIRT6
 296 VNLQW-----TPKDDWAALKL human SIRT7

 510 LGYCDDI-----AAMVAQKCGWTIPHKKWN-----DL yeast SIR2
 516 LSELPTPLHISEDSSSPERTVPQDSSVIATLVDQATNNVNDLEVSSES-SCVEEKPEV mouse SIRT1
 524 LSELPTPLHISEDSSSPERTSPDSSVIVTLLDQAAKSND-DLDVSESKGCMEEKPEV human SIRT1
 321 LGECDDQ-----CLALAEELGWKKELE--D-----LV human SIRT2
 363 LGDVVHG-----VESLVELLGWTEEMR--D-----LV human SIRT3
 301 NSRCGEL-----LPLIDPC human SIRT4
 290 QGPCGTT-----LPEALACHENETVS human SIRT5
 255 HGYVDEV-----MTRLMEHLGLEIPAWDGP---R----VLERALPPL human SIRT6
 312 HGKCDV-----MRLLEAELGLEIPAYS-----RWQDPI human SIRT7

 537 KN-----KN-----FKCQ-- yeast SIR2
 575 QTSRNVENIN--VENPDFKAVGSSTADKNERTSVAETVRKCWPNRLAKEQISKRLEGNQY mouse SIRT1
 583 QTSRNVESIAEQMENPDKNVGSSTGEKNERTSVAGTVRKWCWPNRVAKEQISRRLDGNQY human SIRT1
 346 RR-----EH-----AS---IDAQ-- human SIRT2
 388 QR-----ET-----GK---LDG-- human SIRT3
 314 human SIRT4
 310 human SIRT5
 290 PR-----PPTPKLEPK-- human SIRT6
 341 FS-----LATPLRAGE-- human SIRT7

545	-----EK-----DKGVYVVTSDHPKTL	yeast	SIR2
633	LFVPPNRYIFHGAEVYSDSEDDVLSSSSCGSNSDSGTCQSPSLEEPLSESEIEEFYNGL	mouse	SIRT1
643	LFLPPNRYIFHGAEVYSDSEDDVLSSSSCGSNSDSGTCQSPSLEEPMESEIEEFYNGL	human	SIRT1
356	-----SGAGVPNPS-----TSASPKKSPPPAKDEARTTEREKPQ	human	SIRT2
397	-----PDK	human	SIRT3
314		human	SIRT4
310		human	SIRT5
301	-----EESPTRING-----SIPAGPKQEPCAQHNGSEPASPKRERPTSPA	human	SIRT6
352	-----EGSHSRK-----SLCRSREEAPPGDRGAPLSSAPILGGWFG--	human	SIRT7
562		yeast	SIR2
693	EDDTERPECAGGSGFGADGGDQEVVNEAIATRQELTDVNYPSDKS	mouse	SIRT1
703	EDEPDVPERAGGAGFGTDGDDQEAINAISVKQEVTDNMNYPNKS	human	SIRT1
389		human	SIRT2
399		human	SIRT3
314		human	SIRT4
310		human	SIRT5
341	PHRPPKRVKAKAVPS	human	SIRT6
388	-RGCTKRTRKKVT	human	SIRT7