(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2011 (17.03.2011)

PCT

(10) International Publication Number
WO 2011/031471 A2

(51) International Patent Classification:
C12N 15/62 (2006.01) *C12Q 1/66* (2006.01)
C12N 15/65 (2006.01) *A61P 31/04* (2006.01)
C12N 1/15 (2006.01) *C12R 1/66* (2006.01)

(21) International Application Number:
PCT/US2010/046601

(22) International Filing Date:
25 August 2010 (25.08.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/236,644 25 August 2009 (25.08.2009) US

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(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, CL, 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, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2011/031471 A2

(54) Title: THE FUNGAL SREBP PATHWAY AS A TARGET FOR ANTI-FUNGAL AGENTS

(57) Abstract: Inhibition of Srel activity and/or expression has great potential for antifungal therapeutics. Disclosed herein are novel methods and compositions useful for the identification of novel inhibitors of Srel activity and/or function and the treatment of fungal infections.

The Fungal SREBP Pathway as a Target for Anti-Fungal Agents

RELATED APPLICATIONS

This application claims the benefit of priority to United States Provisional Patent Application serial number 61/236,644, filed August 25, 2009; which is hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT

This invention was made with support provided by the National Institutes of Health (grant R21 AI072186). The Government has certain rights in the invention.

BACKGROUND

Opportunistic fungal pathogens, such as *Cryptococcus neoformans* and *Aspergillus fumigatus,* can cause life threatening infections in immunocompromised patients, including organ transplant recipients and individuals with HIV/AIDS or leukemia.

Several factors have been identified as being required to promote disease, including the ability of the pathogenic cells to grow under conditions of hypoxia. For example, in instances of *Cryptococcus neoformans* infection, yeast cells and/or spores are inhaled and disseminate to the brain where they establish growth and formation of cystic lesions. In the brain, *Cryptococcus neoformans* cells experience limiting oxygen and, in order to continue to grow, the cells must adapt to the hypoxic environment. Chemical compounds that interfere with the ability of the fungus to grow under such conditions therefore have great potential as therapeutic agents.

Clinical treatment of human fungal infections primarily relies on a few types of antifungal agents. Commonly used fungicidal compounds such as amphotericin B, flucytosine and nystatin are capable of curing fungal infections, but also result in severe side effects to the patient. Azole agents, such as fluconazole, which inhibit ergosterol biosynthesis, exhibit fewer side effects, but are only fungistatic, rather than fungicidal. Thus, there exists a need for new methods and compositions for the treatment of fungal infections and/or the identification of novel fungicidal compounds.

SUMMARY

In some embodiments, the instant invention relates to compositions, including an isolated nucleic acid molecule and/or a plasmid, that include a sequence encoding a reporter protein operably linked to the promoter of a gene that is upregulated by Sre1. In certain embodiments the promoter is an ERG25 promoter. In some embodiments, the reporter protein is a fusion protein that includes amino acids 1-30 of the Erg25 protein (Erg25p) or fragments thereof. In certain embodiments, the reporter protein is a fusion protein that includes the Erg25 protein (Erg25p) or a fragment of the Erg25p. In some embodiments, the Erg25p fragment includes amino acids 1-10, 1-20 and/or 1-30 of Erg25p. In other embodiments, the reporter protein includes any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 consecutive amino acids of the Erg25p. In some embodiments, the reporter protein is a fusion protein that includes amino acids 1-10, 1-20 and/or 1-30 of the Erg25p. In some embodiments the reporter protein is a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker.

In some embodiments of the invention, the nucleic acid molecule and/or plasmid also includes a second nucleic acid sequence encoding a second reporter protein that is operably linked to a second promoter. In certain embodiments the second promoter is a constitutive promoter, such as the Histone H3 promoter. In certain embodiments the second reporter protein is a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker.

Certain embodiments of the instant invention relate to a fungal cell that includes a first nucleic acid sequence encoding a first reporter protein operably linked to the promoter of a gene that is upregulated by Sre1. In some embodiments the promoter is an ERG25 promoter. In certain embodiments, the first nucleic acid sequence is integrated into the chromosomal DNA of the fungal cell, while in other embodiments the first nucleic acid sequence is extra-chromosomal. In some embodiments the first reporter protein is a fusion protein that comprises amino acids 1-10, 1-20 and/or 1-30 of Erg25p. In some embodiments the reporter protein is a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker.

In some embodiments of the invention, the fungal cell also includes a second nucleic acid sequence encoding a second reporter protein that is operably linked to a second promoter. In certain embodiments, the second nucleic acid sequence is integrated into the

chromosomal DNA of the fungal cell, while in other embodiments the second nucleic acid sequence is extra-chromosomal. In certain embodiments the second promoter is a constitutive promoter, such as the Histone H3 promoter. In certain embodiments the second reporter protein is a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker.

In some embodiments the fungal cell of the invention is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans.*

Certain aspects of the invention relate to a method of determining whether an agent is an inhibitor of Sre1 expression or activity, which includes contacting a fungal cell with the agent, wherein the fungal cell comprises a first nucleic acid sequence encoding a first reporter protein operably linked to an ERG25 promoter and a second nucleic acid sequence encoding a second reporter protein operably linked to a constitutive promoter and determining the expression level of the first reporter protein compared to the second reporter protein, wherein a reduction of the level of the first reporter protein compared to the second reporter protein indicates that the agent is an inhibitor of Sre1 expression or activity. In certain embodiments, the first and second nucleic acid sequences are integrated into the chromosomal DNA of the fungal cell, while in other embodiments they are extra-chromosomal. In some embodiments the first reporter protein is a fusion protein that comprises amino acids 1-10, 1-20 and/or 1-30 of Erg25p. In some embodiments the first and/or second reporter protein is a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker. In certain embodiments the second promoter is a constitutive promoter, such as the Histone H3 promoter. In some embodiments the fungal cell is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*.

In some embodiments, the invention relates to a method of treating or preventing an infection by a fungus in a subject in need thereof that includes administering to the subject a compound of formula **I - X**, including Dichlorophen, Ursolic acid, Pentifylline, Benzbromarone, Congo Red, Cetalkonium Chloride, Tolonium Chloride, Zinc Undecylenate, Meclocycline Sulfosalicylate Salt, Quinaldine Blue, Evan's Blue, pharmaceutically acceptable salts thereof and derivatives thereof. In certain embodiments the fungus is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*. In some embodiments, the method also includes administering a second antifungal drug, such as an azole drug.

Certain embodiments of the invention relate to a method reducing ERG25 expression in a fungus that includes contacting the fungus with a compound selected from the group consisting of: Dichlorophen, Ursolic acid, Pentifylline, Benzbromarone, Congo Red, Cetalkonium Chloride, Tolonium Chloride, Zinc Undecylenate, Meclocycline Sulfosalicylate Salt, Quinaldine Blue, Evan's Blue, a pharmaceutically acceptable salt thereof and a derivative thereof. In certain embodiments the fungus is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the nucleic acid sequences of the ERG25 promoter and of a fusion reporter protein that contains the first 30 amino acids of the Erg25 protein fused to a codon-optimized GFP reporter.

Figure 2 depicts a map of plasmid pCB61-1.

Figure 3 depicts a map of plasmid pCB54-1.

Figure 4 depicts the nucleic acid sequence of plasmid pCB61-1.

Figure 5 depicts the nucleic acid sequence of plasmid pCB54-1.

Figure 6 depicts the results of a series of experiments that demonstrate that Sre1 and Stp1 are required for fungal growth under hypoxic condition. **Figure 6A** depicts the protein alignment of human Site-2 protease (NCBI refseq ID: NP_056968) and *C. neoformans* Stp1 (BROAD ID: CNAG_05742.2). Putative catalytic residues are underlined. **Figure 6B** depicts a western blot of cell extracts from *stp1*Δ cells that were shifted from 21% oxygen to 3% oxygen for indicated periods of time. Immunoblot analysis was performed on whole cell extracts (40 μg) using anti-Sre1 antiserum. P and N denote the precursor and nuclear forms, respectively. Asterisks indicate non-specific, cross-reacting proteins detected by anti-Sre1 antiserum. **Figure 6C** depicts the results of a quantitative PCR analysis of *C. neoformans* cells from the indicated strains that were grown under ambient (21%) or 3% oxygen for 2 hours. *SRE1* transcript levels were quantified by real time RT-PCR and normalized to that in wild-type cells at 21% oxygen. Error bars represent standard deviation from three biological replicate experiments. **Figure 6D** depicts five-fold serial dilutions of *C. neoformans* cells from the indicated strains that were spotted on rich medium and rich medium containing 0.3 mM $CoCl_2$ and incubated at 30°C for 3 days.

Figure 7 depicts the results of experiments that demonstrate that Sre1 and Stp1 are required for virulence *in vivo*. **Figure 7A** depicts a growth curve for *C. neoformans* serotype A wild-type and *stp1*Δ cells that were grown in YES medium at 37°C. Cell numbers were determined at one hour intervals for 12 hours. Error bars represent one standard deviation from three biological replicates. **Figure 7B** depicts the survival of Female Balb/c mice (n=10/strain) that were infected via tail vein injection with the indicated *C. neoformans* strains.

Figure 8 depicts the results of experiments that demonstrate that azole drugs are fungicidal to *sre1Δ* and *stp1Δ* cells. **Figure 8A** depicts the cell density of wild-type, *sre1Δ* and *stp1Δ* cells that were grown for 48 hours in liquid YES medium. Final cell density was determined using a spectrophotometer (1 OD_{600} = $2x10^7$ cells) at the indicated concentrations of itraconazole (left panel) or 25-thialanosterol (right panel). Error bars represent standard deviation from three biological replicate experiments. **Figure 8B** depicts the percent viability of wild-type, *sre1Δ* and *stp1Δ* cells that were grown for 48 hours in liquid YES medium. Equal numbers of cells were plated and grown on YES medium for 2 days. Colony-forming units were counted and percent viability calculated. **Figure 8C** depicts the percent viability of wild-type, *sre1*Δ and *stp1*Δ cells that were grown for the indicated times in the presence of 2.5 nM itraconazole, 5 nM 25-thialanosterol or DMSO as a vehicle control. Equal numbers of cells were plated and grown on YES medium for 2 days. Error bars represent the standard deviation from three biological replicate experiments.

Figure 9 depicts survival curve of mice that were infected with 5×10^5 wild-type or *sre1*Δ cells and treated with 40 mg/kg of the azole drug fluconazole (flu) or a control (PBS).

DETAILED DESCRIPTION

Cryptococcus neoformans is a basidiomycetous yeast that causes life-threatening meningoencephalitis primarily in immunocompromised patients, particularly individuals with HIV/AIDS. Yeast cells and/or spores are inhaled and disseminate to the brain where they establish growth and formation of cystic lesions. Several factors have been identified as being required to promote disease, including the ability of *C. neoformans* cells to grow at host body temperature, the production of melanin, and the production of a polysaccharide capsule surrounding the cell wall of *C. neoformans* cells. Despite the identification of these

well-known virulence requirements, mechanisms underlying the adaptation of *C. neoformans* cells to the host environment remain poorly understood.

The *C. neoformans* sterol regulatory element-binding protein (SREBP) pathway is required for host adaptation and virulence. *C. neoformans* SREBP, called Sre1, is a membrane-bound transcription factor that stimulates ergosterol production in response to sterol depletion, for example when oxygen-dependent ergosterol synthesis is limited by hypoxia. Sre1 is proteolytically activated under low oxygen conditions and *SRE1* is required for virulence in a mouse model of infection. In this model, *sre1*Δ cells enter the brain but fail to cause lethal infection in the mice, indicating a role for Sre1 in growth or survival in the host tissue.

Most fungal infections are currently treated with drugs that inhibit ergosterol biosynthesis, including the commonly used azole class of drugs. Azoles have been shown to be effective against a wide-range of pathogenic fungi, including species of *Cryptococcus*, *Candida*, *Aspergillus*, *Coccidioides*, and *Histoplasma*, but, as currently used, are fungistatic, rather than fungicidal.

As Sre1 regulates genes encoding ergosterol biosynthetic enzymes, *SRE1* is required for growth in the presence of low levels of azoles. Importantly, SREBP displays a similar requirement in *Aspergillus fumigatus, Aspergillus nidulans, Magnaporthe grisea* and *Candida albicans*. Sre1, or regulators of Sre1, are therefore promising targets for antifungal drug design due to the fact that Sre1 inhibitors display synergistic effects when used in combination with current antifungal compounds.

Thus, there exists a great need for novel methods and compositions for the identification of inhibitors of Sre1 expression and/or activity, such as are provided herein. For example, in some embodiments, the invention relates to nucleic acid molecules, plasmids and fungal cells useful in screening compound libraries for inhibitors of Sre1 expression and/or activity, while in other embodiments the invention relates to methods of using compounds identified in one such screen for the prevention and/or treatment of fungal infections.

Definitions

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles "*a*" and "*an*," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

As used herein, the term "*expression*" means any functions and steps by which a gene's coded information is converted into structures present and operating in a cell.

As used herein, the term "*isolated*" refers to the state in which substances (e.g., polypeptides, polynucleotides or viruses) are free or substantially free of material with which they are naturally associated such as other polypeptides or polynucleotides with which they are found in their natural environment or the environment in which they are prepared (*e.g.,* cell culture). Polypeptides, polynucleotides or viruses can be formulated with diluents or adjuvants and still be considered "isolated" - for example, polypeptides, polynucleotides or viruses can be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy.

As used herein, the terms "*nucleic acid*," "*polynucleotide*," "*polynucleotide sequence*" and "*nucleic acid sequence*" refer to single-stranded or double-stranded deoxyribonucleotide or ribonucleotide polymers, or chimeras or analogues thereof. As used herein, the term optionally includes polymers of analogs of naturally occurring nucleotides having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (*e.g.,* peptide nucleic acids).

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 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.

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 from one organ, or portion of the body, to another organ, or portion of the body. 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 term "*pharmaceutically-acceptable salts*" is art-recognized and refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds, including, for example, those contained in compositions described herein.

As used herein, the term "*preventing*" or "*prevention*" refers to delaying or forestalling the onset, development or progression of a condition or disease for a period of time, including weeks, months, or years.

As used herein, the term "*reporter protein*" refers to conveniently detectable proteins that are not normally present in the research system being used. For example, in the screens described herein, endogenous fungal proteins are not suitable as reporters. Examples of reporter proteins include fluorescent proteins such as green fluorescent protein (GFP) and DsRED, proteins that are able to catalyze light-producing reactions, such as luciferase, proteins that catalyze reactions that result in a colored product, such as beta-glucuronidase, selectable marker proteins, which are required for cell survival and/or growth under certain culture conditions and counter-

selectable marker proteins, such as URA5, which inhibit cell survival and/or growth under certain culture conditions.

As used herein, the term "*subject*" means a human or non-human animal selected for treatment or therapy.

As used herein, the phrase "*subject in need thereof*" means a subject identified as in need of a therapy or treatment of the invention.

The terms "*test compound*" and "*agent*" are used herein to denote a chemical compound, a small molecule, a mixture 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. Test compounds and agents may be identified as having a particular activity by screening assays described herein below.

As used herein, the term "*treating*" a disease in a subject or "*treating*" a subject having or suspected of having a disease refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of an agent, such that at least one symptom of the disease is decreased or prevented from worsening.

As used herein, the term "*vector*" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "*plasmid*", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced. Other 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 a nucleic acid incorporated therein. Such vectors are referred to herein as "*expression vectors*". Typically, the nucleic acid to be expressed is "*operably linked*" to a transcriptional control element, such as a promoter and/or an enhancer, and is therefore subject to transcription regulatory control by the transcriptional control element.

The definition of each expression, *e.g.,* alkyl, m, n, and the like, when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

It will be understood that "*substitution*" or "*substituted with*" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction.

The term "*substituted*" is also contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein below. The permissible substituents may be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The term "*lower*" when appended to any of the groups listed below indicates that the group contains less than seven carbons (*i.e.* six carbons or less). For example "lower alkyl" refers to an alkyl group containing 1-6 carbons, and "lower alkenyl" refers to an alkyenyl group containing 2-6 carbons.

The term "*saturated*," as used herein, pertains to compounds and/or groups which do not have any carbon-carbon double bonds or carbon-carbon triple bonds.

The term "*unsaturated*," as used herein, pertains to compounds and/or groups which have at least one carbon-carbon double bond or carbon-carbon triple bond.

The term "*aliphatic,*" as used herein, pertains to compounds and/or groups which are linear or branched, but not cyclic (also known as "acyclic" or "open-chain" groups).

The term "*cyclic*," as used herein, pertains to compounds and/or groups which have one ring, or two or more rings (e.g., spiro, fused, bridged).

The term "*aromatic*" refers to a planar or polycyclic structure characterized by a cyclically conjugated molecular moiety containing 4n+2 electrons, wherein n is the absolute value of an integer. Aromatic molecules containing fused, or joined, rings also are referred

to as bicylic aromatic rings. For example, bicyclic aromatic rings containing heteroatoms in a hydrocarbon ring structure are referred to as bicyclic heteroaryl rings.

The term "*hydrocarbon*" as used herein refers to an organic compound consisting entirely of hydrogen and carbon.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover.

The term "*heteroatom*" as used herein is art-recognized and refers to an atom of any element other than carbon or hydrogen. Illustrative heteroatoms include boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The term "*alkyl*" means an aliphatic or cyclic hydrocarbon radical containing from 1 to 12 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 2-methylcyclopentyl, and 1-cyclohexylethyl.

The term "*alkylene*" is art-recognized, and as used herein pertains to a bidentate moiety obtained by removing a hydrogen atom from an alkyl group, as defined above.

The term "*alkenyl*" as used herein means a straight or branched chain hydrocarbon radical containing from 2 to 10 carbons and containing at least one carbon-carbon double bond formed by the removal of two hydrogens. Representative examples of alkenyl include, but are not limited to, ethenyl, 2-propenyl, 2-methyl-2-propenyl, 3-butenyl, 4-pentenyl, 5-hexenyl, 2-heptenyl, 2-methyl-1-heptenyl, and 3-decenyl.

The term "*alkynyl*" as used herein means a straight or branched chain hydrocarbon radical containing from 2 to 10 carbon atoms and containing at least one carbon-carbon triple bond. Representative examples of alkynyl include, but are not limited, to acetylenyl, 1-propynyl, 2-propynyl, 3-butynyl, 2-pentynyl, and 1-butynyl.

The term "*carbocyclyl*" as used herein means monocyclic or multicyclic (e.g., bicyclic, tricyclic, etc.) hydrocarbon radical containing from 3 to 12 carbon atoms that is completely saturated or has one or more unsaturated bonds, and for the avoidance of doubt, the degree of unsaturation does not result in an aromatic ring system (e.g. phenyl). Examples of carbocyclyl groups include 1-cyclopropyl, 1-cyclobutyl, 2-cyclopentyl, 1-cyclopentenyl, 3-cyclohexyl, 1-cyclohexenyl and 2-cyclopentenylmethyl.

The term "*heterocyclyl*", as used herein refers to a radical of a non-aromatic, ring systems, including, but not limited to, monocyclic, bicyclic and tricyclic rings, which can be completely saturated or which can contain one or more units of unsaturation, for the avoidance of doubt, the degree of unsaturation does not result in an aromatic ring system, and have 3 to 12 atoms including at least one heteroatom, such as nitrogen, oxygen, or sulfur. For purposes of exemplification, which should not be construed as limiting the scope of this invention, the following are examples of heterocyclic rings: azepines, azetidinyl, morpholinyl, oxopiperidinyl, oxopyrrolidinyl, piperazinyl, piperidinyl, pyrrolidinyl, quinicludinyl, thiomorpholinyl, tetrahydropyranyl and tetrahydrofuranyl. The heterocyclyl groups of the invention are substituted with 0, 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of alkyl, alkenyl, alkynyl, halo, haloalkyl, fluoroalkyl, hydroxy, alkoxy, alkyenyloxy, alkynyloxy, carbocycyloxy, heterocycyloxy, haloalkoxy, fluoroalkyloxy, sulfhydryl, alkylthio, haloalkylthio, fluoroalkylthio, alkyenylthio, alkynylthio, sulfonic acid, alkylsulfonyl, haloalkylsulfonyl, fluroralkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, alkoxysulfonyl, haloalkoxysulfonyl, fluroralkoxysulfonyl, alkenyloxysulfonyl, alkynyloxysulfony, aminosulfonyl, sulfinic acid, alkylsulfinyl, haloalkylsulfinyl, fluroralkylsulfinyl, alkenylsulfinyl, alkynylsulfinyl, alkoxysulfinyl, haloalkoxysulfinyl, fluroralkoxysulfinyl, alkenyloxysulfinyl, alkynyloxysulfiny, aminosulfinyl, formyl, alkylcarbonyl, haloalkylcarbonyl, fluoroalkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, carboxy, alkoxycarbonyl, haloalkoxycarbonyl, fluoroalkoxycarbonyl, alkenyloxycarbonyl, alkynyloxycarbonyl, alkylcarbonyloxy, haloalkylcarbonyloxy, fluoroalkylcarbonyloxy, alkenylcarbonyloxy, alkynylcarbonyloxy, alkylsulfonyloxy, haloalkylsulfonyloxy, fluroralkylsulfonyloxy, alkenylsulfonyloxy, alkynylsulfonyloxy, haloalkoxysulfonyloxy, fluroralkoxysulfonyloxy, alkenyloxysulfonyloxy, alkynyloxysulfonyloxy, alkylsulfinyloxy, haloalkylsulfinyloxy, fluroralkylsulfinyloxy, alkenylsulfinyloxy, alkynylsulfinyloxy, alkoxysulfinyloxy, haloalkoxysulfinyloxy, fluroralkoxysulfinyloxy, alkenyloxysulfinyloxy, alkynyloxysulfinyloxy, aminosulfinyloxy, amino, amido, aminosulfonyl, aminosulfinyl, cyano, nitro, azido, phosphinyl, phosphoryl, silyl, silyloxy, and any of said substiuents bound to the heterocyclyl group through an alkylene moiety (e.g. methylene).

The term "*aryl*," as used herein means a phenyl group, naphthyl or anthracenyl group. The aryl groups of the present invention are substituted with 0, 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of alkyl, alkenyl, alkynyl,

halo, haloalkyl, fluoroalkyl, hydroxy, alkoxy, alkyenyloxy, alkynyloxy, carbocycyloxy, heterocycyloxy, haloalkoxy, fluoroalkyloxy, sulfhydryl, alkylthio, haloalkylthio, fluoroalkylthio, alkyenylthio, alkynylthio, sulfonic acid, alkylsulfonyl, haloalkylsulfonyl, fluroralkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, alkoxysulfonyl, haloalkoxysulfonyl, fluroralkoxysulfonyl, alkenyloxysulfonyl, alkynyloxysulfony, aminosulfonyl, sulfinic acid, alkylsulfinyl, haloalkylsulfinyl, fluroralkylsulfinyl, alkenylsulfinyl, alkynylsulfinyl, alkoxysulfinyl, haloalkoxysulfinyl, fluroralkoxysulfinyl, alkenyloxysulfinyl, alkynyloxysulfiny, aminosulfinyl, formyl, alkylcarbonyl, haloalkylcarbonyl, fluoroalkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, carboxy, alkoxycarbonyl, haloalkoxycarbonyl, fluoroalkoxycarbonyl, alkenyloxycarbonyl, alkynyloxycarbonyl, alkylcarbonyloxy, haloalkylcarbonyloxy, fluoroalkylcarbonyloxy, alkenylcarbonyloxy, alkynylcarbonyloxy, alkylsulfonyloxy, haloalkylsulfonyloxy, fluroralkylsulfonyloxy, alkenylsulfonyloxy, alkynylsulfonyloxy, haloalkoxysulfonyloxy, fluroralkoxysulfonyloxy, alkenyloxysulfonyloxy, alkynyloxysulfonyloxy, alkylsulfinyloxy, haloalkylsulfinyloxy, fluroralkylsulfinyloxy, alkenylsulfinyloxy, alkynylsulfinyloxy, alkoxysulfinyloxy, haloalkoxysulfinyloxy, fluroralkoxysulfinyloxy, alkenyloxysulfinyloxy, alkynyloxysulfinyloxy, aminosulfinyloxy, amino, amido, aminosulfonyl, aminosulfinyl, cyano, nitro, azido, phosphinyl, phosphoryl, silyl, silyloxy, cyclic acetal, and any of said substituents bound to the heterocyclyl group through an alkylene moiety (e.g. methylene).

The term "*heteroaryl*" as used herein refers to a radical of an aromatic ring, including, but not limited to, monocyclic, bicyclic and tricyclic rings, which has 3 to 12 atoms including at least one heteroatom, such as nitrogen, oxygen, or sulfur. For purposes of exemplification, which should not be construed as limiting the scope of this invention: azaindolyl, benzo(b)thienyl, benzimidazolyl, benzofuranyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, benzotriazolyl, benzoxadiazolyl, furanyl, imidazolyl, imidazopyridinyl, indolyl, indolinyl, indazolyl, isoindolinyl, isoxazolyl, isothiazolyl, isoquinolinyl, oxadiazolyl, oxazolyl, purinyl, pyranyl, pyrazinyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrrolyl, pyrrolo[2,3-d]pyrimidinyl, pyrazolo[3,4-d]pyrimidinyl, quinolinyl, quinazolinyl, triazolyl, thiazolyl, thiophenyl, tetrahydroindolyl, tetrazolyl, thiadiazolyl, thienyl, thiomorpholinyl, triazolyl or tropanyl. The heteroaryl groups of the invention are substituted with 0, 1, 2, 3, 4 or 5 substituents independently selected from the group consisting of alkyl, alkenyl, alkynyl, halo, haloalkyl, fluoroalkyl, hydroxy, alkoxy, alkyenyloxy, alkynyloxy, carbocycyloxy, heterocycyloxy, haloalkoxy, fluoroalkyloxy,

sulfhydryl, alkylthio, haloalkylthio, fluoroalkylthio, alkyenylthio, alkynylthio, sulfonic acid, alkylsulfonyl, haloalkylsulfonyl, fluroralkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, alkoxysulfonyl, haloalkoxysulfonyl, fluroralkoxysulfonyl, alkenyloxysulfonyl, alkynyloxysulfony, aminosulfonyl, sulfinic acid, alkylsulfinyl, haloalkylsulfinyl, fluroralkylsulfinyl, alkenylsulfinyl, alkynylsulfinyl, alkoxysulfinyl, haloalkoxysulfinyl, fluroralkoxysulfinyl, alkenyloxysulfinyl, alkynyloxysulfiny, aminosulfinyl, formyl, alkylcarbonyl, haloalkylcarbonyl, fluoroalkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, carboxy, alkoxycarbonyl, haloalkoxycarbonyl, fluoroalkoxycarbonyl, alkenyloxycarbonyl, alkynyloxycarbonyl, alkylcarbonyloxy, haloalkylcarbonyloxy, fluoroalkylcarbonyloxy, alkenylcarbonyloxy, alkynylcarbonyloxy, alkylsulfonyloxy, haloalkylsulfonyloxy, fluroralkylsulfonyloxy, alkenylsulfonyloxy, alkynylsulfonyloxy, haloalkoxysulfonyloxy, fluroralkoxysulfonyloxy, alkenyloxysulfonyloxy, alkynyloxysulfonyloxy, alkylsulfinyloxy, haloalkylsulfinyloxy, fluroralkylsulfinyloxy, alkenylsulfinyloxy, alkynylsulfinyloxy, alkoxysulfinyloxy, haloalkoxysulfinyloxy, fluroralkoxysulfinyloxy, alkenyloxysulfinyloxy, alkynyloxysulfinyloxy, aminosulfinyloxy, amino, amido, aminosulfonyl, aminosulfinyl, cyano, nitro, azido, phosphinyl, phosphoryl, silyl, silyloxy, and any of said subsituents bound to the heteroaryl group through an alkylene moiety (e.g. methylene).

The term "*halo*" or "halogen" means -Cl, -Br, -I or -F.

The term "*haloalkyl*" means an alkyl group, as defined herein, wherein at least one hydrogen is replaced with a halogen, as defined herein. Representative examples of haloalkyl include, but are not limited to, chloromethyl, 2-fluoroethyl, trifluoromethyl, pentafluoroethyl, and 2-chloro-3-fluoropentyl.

The term "*fluoroalkyl*" means an alkyl group, as defined herein, wherein all the hydrogens are replaced with fluorines.

The term "*hydroxy*" as used herein means an -OH group.

The term "*alkoxy*" as used herein means an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, tert-butoxy, pentyloxy, and hexyloxy. The terms "alkyenyloxy", "alkynyloxy", "carbocycyloxy", and "heterocycyloxy" are likewise defined.

The term "*haloalkoxy*" as used herein means an alkoxy group, as defined herein, wherein at least one hydrogen is replaced with a halogen, as defined herein. Representative

examples of haloalkoxy include, but are not limited to, chloromethoxy, 2-fluoroethoxy, trifluoromethoxy, and pentafluoroethoxy. The term "fluoroalkyloxy" is likewise defined.

The term "*aryloxy*" as used herein means an aryl group, as defined herein, appended to the parent molecular moiety through an oxygen. The term "*heteroaryloxy*" as used herein means a heteroaryl group, as defined herein, appended to the parent molecular moiety through an oxygen. The terms "heteroaryloxy" is likewise defined.

The term "*arylalkoxy*" or "*arylalkyloxy*" as used herein means an arylalkyl group, as defined herein, appended to the parent molecular moiety through an oxygen. The term "heteroarylalkoxy" is likewise defined. Representative examples of aryloxy and heteroarylalkoxy include, but are not limited to, 2-chlorophenylmethoxy, 3-trifluoromethyl-phenylethoxy, and 2,3-dimethylpyridinylmethoxy.

The term "*sulfhydryl*" or "*thio*" as used herein means a -SH group.

The term "*alkylthio*" as used herein means an alkyl group, as defined herein, appended to the parent molecular moiety through a sulfur. Representative examples of alkylthio include, but are not limited, methylthio, ethylthio, tert-butylthio, and hexylthio. The terms "haloalkylthio", "fluoroalkylthio", "alkyenylthio", "alkynylthio", "carbocycylthio", and "heterocycylthio" are likewise defined.

The term "*arylthio*" as used herein means an aryl group, as defined herein, appended to the parent molecular moiety through an sulfur. The term "heteroarylthio" is likewise defined.

The term "*arylalkylthio*" or "*aralkylthio*" as used herein means an arylalkyl group, as defined herein, appended to the parent molecular moiety through an sulfur. The term "heteroarylalkylthio" is likewise defined.

The term "*sulfonyl*" as used herein refers to $-S(=O)_2-$ group.

The term "*sulfonic acid*" as used herein refers to $-S(=O)_2OH$.

The term "*alkylsulfonyl*" as used herein means an alkyl group, as defined herein, appended to the parent molecular moiety through a sulfonyl group, as defined herein. Representative examples of alkylsulfonyl include, but are not limited to, methylsulfonyl and ethylsulfonyl. The terms "haloalkylsulfonyl", "fluroralkylsulfonyl", "alkenylsulfonyl", "alkynylsulfonyl", "carbocycylsulfonyl", "heterocycylsulfonyl", "arylsulfonyl", "aralkylsulfonyl", "heteroarylsulfonyl" and "heteroaralkylsulfonyl" are likewise defined.

The term "*alkoxysulfonyl*" as used herein means an alkoxy group, as defined herein, appended to the parent molecular moiety through a sulfonyl group, as defined herein. Representative examples of alkoxysulfonyl include, but are not limited to, methoxysulfonyl, ethoxysulfonyl and propoxysulfonyl. The terms "haloalkoxysulfonyl", "fluroralkoxysulfonyl", "alkenyloxysulfonyl", "alkynyloxysulfonyl", "carbocycyloxysulfonyl", "heterocycyloxysulfonyl", "aryloxysulfonyl", "aralkyloxysulfonyl", "heteroaryloxysulfonyl" and "heteroaralkyloxysulfonyl" are likewise defined.

The term "*oxy*" refers to a -O- group.

The term "*carbonyl*" as used herein means a -C(=O)- group.

The term "*alkylcarbonyl*" as used herein means an alkyl group, as defined herein, appended to the parent molecular moiety through a carbonyl group, as defined herein. Representative examples of alkylcarbonyl include, but are not limited to, acetyl, 1-oxopropyl, 2,2-dimethyl-1-oxopropyl, 1-oxobutyl, and 1-oxopentyl. The terms "haloalkylcarbonyl", "fluoroalkylcarbonyl", "alkenylcarbonyl", "alkynylcarbonyl", "carbocycylcarbonyl", "heterocycylcarbonyl", "arylcarbonyl", "aralkylcarbonyl", "heteroarylcarbonyl", and "heteroaralkylcarbonyl" are likewise defined.

The term "*carboxy*" as used herein means a $-CO_2H$ group.

The term "*alkoxycarbonyl*" as used herein means an alkoxy group, as defined herein, appended to the parent molecular moiety through a carbonyl group, as defined herein. Representative examples of alkoxycarbonyl include, but are not limited to, methoxycarbonyl, ethoxycarbonyl, and tert-butoxycarbonyl. The terms "haloalkoxycarbonyl", "fluoroalkoxycarbonyl", "alkenyloxycarbonyl", "alkynyloxycarbonyl", "carbocycyloxycarbonyl", "heterocycyloxycarbonyl", "aryloxycarbonyl", "aralkyloxycarbonyl", "heteroaryloxycarbonyl", and "heteroaralkyloxycarbonyl" are likewise defined.

The term "*amino*" as used herein refers to $-NH_2$ and substituted derivatives thereof wherein one or both of the hydrogens are independently replaced with substituents selected from the group consisting of alkyl, haloalkyl, fluoroalkyl, alkenyl, alkynyl, carbocycyl, heterocycyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkylcarbonyl, haloalkylcarbonyl, fluoroalkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, carbocycylcarbonyl, heterocycylcarbonyl, arylcarbonyl, aralkylcarbonyl, heteroarylcarnbonyl,

heteroaralkylcarbonyl and the sufonyl and sulfinyl groups defined above; or when both hydrogens together are replaced with an alkylene group (to form a ring which contains the nitrogen). Representative examples include, but are not limited to methylamino, acetylamino, and dimethylamino.

The term "*amido*" as used herein means an amino group, as defined herein, appended to the parent molecular moiety through a carbonyl.

The term "*cyano*" as used herein means a -C≡N group.

The term "*nitro*" as used herein means a $-NO_2$ group.

The term "*azido*" as used herein means a $-N_3$ group.

The abbreviations Me, Et, Ph, Bn, Tf, Nf, Ts, and Ms represent methyl, ethyl, phenyl, benzyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled *Standard List of Abbreviations.*

Nucleic Acids

In certain embodiments, the invention relates to isolated nucleic acid molecules that are useful for identifying inhibitors of the activity or expression of Sre1. As used herein, the term "nucleic acid molecule" is used interchangeably with "polynucleotide" and includes DNA molecules and RNA molecules and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule or polynucleotide can be single-stranded or double-stranded.

In some embodiments, the invention relates to an isolated nucleic acid molecule, plasmid or fungal cell that comprises a sequence encoding a reporter protein operably linked to the promoter of a gene that is upregulated by Sre1. Exemplary genes that are upregulated by Sre1 in *C. neoformans* are provided in Table 1. The SREBP pathway is conserved across many species of fungus, including *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* and *Candida albicans*. It is therefore expected that the species-specific homologs of the genes listed in Table 1 that are found in

such fungal species would also be upregulated by Sre1 or the species-specific homolog of Sre1.

Table 1. Genes upregulated by Sre1.

Gene ID	Description
134106944	Sterol-C5-desaturase *(ERG3)*
134116335	bHLH transcription factor *(SRE1)*
134109756	C-4 methyl sterol oxidase (*ERG25)*
134106030	Lanosterol 14 alpha-demethylase (*ERG11)*
134108153	Sterol methyltransferase (*ERG6)*
134112803	C-22 sterol desaturase (*ERG5)*
134113057	Metalloprotease
134109206	Hydroxymethylglutaryl-CoA synthase (*ERG13)*
134109328	Sterol C-24 reductase (*ERG4)*
134111301	High affinity copper transporter (*CTR4*)
58259314	C-8 sterol isomerase (*ERG2)*
58265871	squalene monooxygenase (*ERG1)*
58262033	putative uridine transporter
58266171	phospholipid transporter
58267325	transmembrane transporter Liz1p
58261967	amino acid transporter
58266623	amino acid transporter
58258402	ammonium transporter *MEP1*
58271575	putative allantoate transporter
58266317	urea transporter
58268217	putative allantoate transporter
58266129	Aldehyde dehydrogenase (ALDDH)
58266619	nitrogen metabolism-related protein
58270417	urease
58258158	vacuole protein
58271089	uricase (urate oxidase)
58258744	NADH dehydrogenase
58258744	allantoinase
58259905	cytoplasm protein
58263067	carboxypeptidase s precursor
58261627	putative transcription factor
58268253	dihydropyrimidinase
58261493	purine-cytosine permease
58265449	glycerate-and formate-dehydrogenase
58262261	malate dehydrogenase (oxaloacetate-decarboxylating)
58262251	hydrolase
58264775	beta-glucosidase
58260321	glutamate synthase (NADH)
58267313	general amidase
58260357	L-serine ammonia-lyase
58270611	malate synthase
58264499	glutamate dehydrogenase ($NADP^+$)

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The promoters of the genes listed in Table 1 and their homologs are known in the art or can be determined through routine experimentation. For example, where the specific boundaries of the promoter sequence has not been identified, the approximately 2000, 1500, 1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100 or 50 nucleotides upstream of that gene's transcriptional start site in the fungal genome would likely include that gene's promoter and therefore could be operably linked to the sequence encoding a reporter protein in the nucleic acid molecules of the invention.

In some embodiments, the Sre1 inducible promoters disclosed herein do not need to be identical to the sequences disclosed herein in order to retain their function in the instant invention. Thus, for example, nucleic acid sequences that are at least about 50%, 60%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a promoter sequence described herein are encompassed by the invention and are considered "a promoter of a gene that is upregulated by Sre1" so long as Sre1 is able to induce expression of an operably linked sequence to that promoter.

In some embodiments, the promoter that is upregulated by Sre1 is the ERG25 promoter. An ERG25 promoter sequence is provided, for example, as SEQ ID NO: 1 and in Figure 1. In certain embodiments, the ERG25 promoter of the invention has a sequence that is least about 50%, 60%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In some embodiments, the ERG25 promoter of the invention has a sequence that includes 900, 800, 700, 600, 500, 450, 400, 350, 300, 250, 200, 150, 100, 75, or 25 consecutive nucleotides of SEQ ID NO: 1.

In some embodiments, the nucleic acid molecule of the invention includes a constitutive promoter operably linked to a sequence encoding a reporter protein. Constitutive promoters are well known in the art and include any promoter that constitutively drives expression of the gene to which it is operably linked under standard culture conditions. In certain embodiments of the invention, the constitutive promoter is the Histone H3 promoter.

In certain embodiments, the nucleic acid molecules of the invention include sequences encoding reporter proteins. Reporter proteins useful in the invention include, for example, any conveniently detectable protein that is not normally present in the research system being used. For example, if the nucleic acid of the invention is being used in *C. neoformans*,

endogenous *C. neoformans* proteins are not suitable reporter proteins. Examples of reporter proteins include fluorescent proteins such as green fluorescent protein (GFP) and DsRED, proteins that are able to catalyze light-producing reactions, such as firefly or renilla luciferase, proteins that catalyze reactions that result in a colored product, such as beta-glucuronidase, selectable marker proteins, which are required for cell survival and/or growth under certain culture conditions and counter-selectable marker proteins, such as Ura5, which inhibit cell survival and/or growth of otherwise Ura5 deficient cells under certain culture conditions.

In certain embodiments, the expression of the reporter protein is regulated by the promoter of a gene that is upregulated by Sre1. In some embodiments, the reporter gene is expressed as a fusion with all of or a fragment of the protein encoded by the Sre1 upregulated gene. For example, in certain embodiments of the invention in which the ERG25 promoter drives expression of the reporter gene, the reporter gene is expressed as a fusion protein that includes all of or a portion of the Erg25 protein. In some embodiments the reporter protein is fused to all of the protein encoded by the Sre1 upregulated gene. In certain embodiments, the reporter protein is fused a fragment of the protein encoded by the Sre1 upregulated gene that includes the first 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 amino acids of the protein encoded by the Sre1 upregulated gene. For example, in certain embodiments of the invention the reporter protein is fused to the first 30 amino acids of the Erg25 protein: AAQIAFFFVFEDTFHYWAHRALHYGPLYKH (SEQ ID NO: 2).

A nucleic acid molecule of the present invention or a portion thereof can be isolated and/or amplified using standard molecular biology techniques. For example, a nucleic acid molecule encompassing all or a portion of a promoter of a gene that is upregulated by Sre1 can be isolated by polymerase chain reaction (PCR) using synthetic oligonucleotide primers.

In certain embodiments, the invention relates to plasmids comprising a nucleic acid molecule of the invention. In certain embodiments, such plasmids further comprise an origin of replication and/or one or more antibiotic resistance genes. Maps of exemplary plasmids of the invention are provided in Figures 2 and 3. Nucleic acid sequences of the plasmids mapped in Figures 2 and 3 are provided in Figures 4 and 5, respectfully.

A nucleic acid molecule of the invention can, for example, be cloned into a plasmid of the invention or other vector and amplified in an appropriate host cell (*e.g.,* a bacterial

cell or a fungal cell), or can be amplified using a RNA or DNA template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, nucleic acid molecules of the invention can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA or RNA synthesizer.

In some embodiments, the nucleic acid molecule of the invention hybridizes under stringent conditions to a nucleic acid sequence described herein (*e.g.*, the promoter of a gene that is upregulated by Sre1). As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), chapters 7, 9 and 11. A non-limiting example of stringent hybridization conditions includes hybridization in 4x or 6x sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4x SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1x SSC, at about 65-70°C. Additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like.

Fungal Cells

Certain embodiments of the invention relate to fungal cells comprising a nucleic acid molecule of the invention. For example, in certain embodiments, the fungal cells of the invention comprise a nucleic acid sequence encoding the promoter of a gene upregulated by Sre1 operably linked to a reporter protein. In certain embodiments the nucleic acid molecule of the invention is integrated into the chromosomal DNA of the fungal cell of the invention, while in some embodiments the nucleic acid of the invention is extrachromasomal.

Contacting the fungal cells of the invention with an agent that inhibits expression or activity of Sre1 results in the fungal cells expressing a reduced level of a reporter protein.

The fungal cells of the invention are therefore useful, for example, in assays for determining whether a particular agent inhibits the activity or expression of Sre1, such as in high throughput compound screens.

In certain embodiments, the reporter protein is a counter-selective marker. In such embodiments, expression of the reporter gene inhibits cell growth in the presence of a particular chemical compound. Inhibition of Sre1 expression or activity in such a cell would reduce transcription of the counter-selective marker and thus permit cell growth. An inhibitor of Sre1 activity or expression could be identified using such cell by, for example, screening for increased cell density and growth. In certain embodiments, the counter-selectable reporter gene is URA5, which inhibits cell growth in the presence of 5-Fluoroorotic Acid.

In some embodiments, the fungal cell of the invention further comprises a second promoter operably linked to a nucleic acid sequence that encodes a second reporter protein. In certain embodiments, the second promoter is a constitutive promoter, such as the Histone H3 promoter. The second reporter protein can be any reporter protein that is distinguishable from the first reporter protein that is encoded by the nucleic acid sequence operably linked to the Sre1 inducible promoter. The second reporter protein can be used, for example, to control for variations in fungal cell concentration and to rule out non-specific inhibitors of transcription and/or translation when the fungal cells are used to identify inhibitors of the activity or expression of Sre1.

In certain embodiments, a fungal cell of the invention can be any fungal cell that has an intact SREBP pathway. For example, the fungal cell of the invention can be *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*. All references to particular fungal species herein encompass all related species, strains and naturally occurring genetic variants of that species. For example, the phrase "*Cryptococcus neoformans*" encompasses *Cryptococcus neoformans* and all related species, strains and naturally occurring genetic variants of *Cryptococcus neoformans*.

Compound Screens

Certain embodiments of the invention relate to methods of determining whether an agent is an inhibitor of Sre1 expression or activity. Such methods are useful, for example, to identify novel antifungal compounds.

In certain embodiments, the methods of the invention include the steps of contacting a fungal cell of the invention with a test compound or a combination of test compounds and detecting the expression of one or more reporter proteins. Compounds that reduce the expression of a reporter protein encoded by a nucleic acid sequence operably linked to a Sre1 inducible promoter are likely inhibitors of Sre1 activity or expression.

In certain embodiments, the expression of this first reporter protein is compared to the expression of a second reporter protein encoded by a nucleic acid sequence operably linked to a constitutive promoter. In such embodiments, reduced expression of the first reporter protein compared to the second reporter protein in the presence of a particular compound indicates that that compound is likely an inhibitor Sre1 activity or expression.

The compound screening methods of the present invention can be practiced manually, can be automated or can be semi-automated. The methods of the invention can be used, for example, to identify whether a single candidate compound is an inhibitor of Sre1 expression or activity or can be used to screen libraries of compounds.

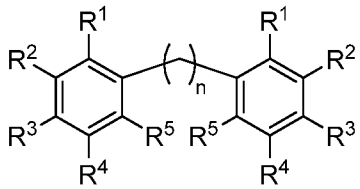
Agents useful in the methods of the present invention may be obtained from any available source, including systematic libraries of natural and/or synthetic compounds. Agents may also be obtained by any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.,* Zuckermann *et al.*, 1994, *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Antifungal Agents and Pharmaceutical Compositions Thereof

In certain embodiments, the invention relates to inhibitors of Sre1 activity and/or expression identified using the screening methods of the instant invention.

In certain embodiments, the invention relates to a compound of formula **I**



I

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein

n is 0, 1, or 2;

R^1, R^2, R^3, R^4, and R^5 represent hydrogen, halo, hydroxy, alkyl, amino, or cyano;

at least one of R^1, R^2, R^3, R^4, or R^5 is halo; and

at least one of R^1, R^2, R^3, R^4, or R^5 is hydroxy.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound is symmetrically substituted.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 1.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least one of R^1, R^2, R^3, R^4, or R^5 is chloro.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is halo.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is chloro.

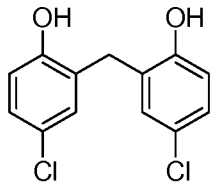
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is hydroxy.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2, R^3, and R^5 are hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **I** is not dichlorophen or 

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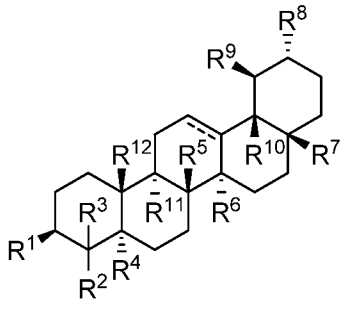
In certain embodiments, the invention relates to a compound of formula **II**



II

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, and R^{12} are hydrogen, halo, hydroxy, alkyl, amino, cyano, or $-CO_2R^{13}$;

R^{13} is hydrogen or alkyl;

 represents a double bond or a single bond;

at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, or R^{12} is hydroxy;

at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, or R^{12} is alkyl; and

at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, or R^{12} is $-CO_2R^{13}$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, or R^{12} is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least one of R^2, R^3, R^5, R^6, R^8, R^9, or R^{12} is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least one of R^2, R^3, R^5, R^6, R^8, R^9, or R^{12} is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is hydroxy.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 and R^3 are the same.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^6 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^6 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^7 is $-CO_2R^{13}$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^7 is $-CO_2H$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^8 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^8 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^9 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^9 is methyl.

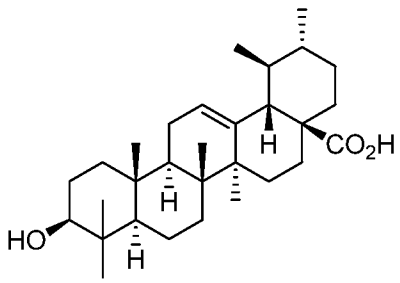
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{10} is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{11} is hydrogen.

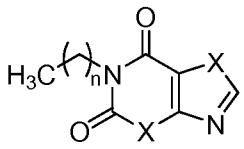
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{12} is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{12} is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein represents a double bond.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **II** is not ursolic acid or



In certain embodiments, the invention relates to a compound of formula **III**



III

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

n is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

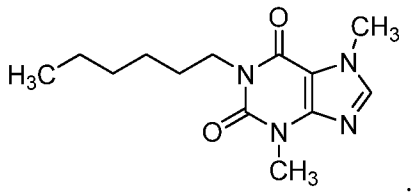
X is -O-, or -N(R^1)-; and

R^1 is hydrogen or alkyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 4, 5, or 6. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 5.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X is -N(R^1)-. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X is -NH-. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X is -N(CH_3)-.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **III** is not pentifylline or



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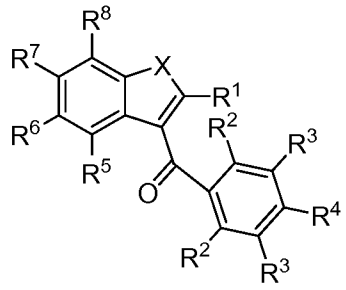
In certain embodiments, the invention relates to a compound of formula **IV**



IV

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein

X is -O-, or -N(R^1)-

R^1 is hydrogen or alkyl;

R^2 is hydrogen, halo, hydroxy, alkyl, amino, or cyano;

R^3 is halo;

R^4 is halo, hydroxy, or amino;

R^5, R^6, R^7, and R^8 are hydrogen, halo, hydroxy, alkyl, amino, or cyano.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X is -O-.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is ethyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is bromo.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is hydroxy or amino. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is hydroxy.

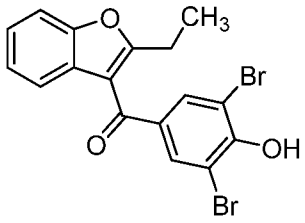
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^6 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^7 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^8 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **IV** is not benzbromarone or



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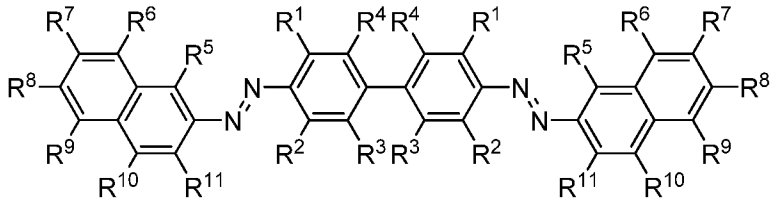
In certain embodiments, the invention relates to a compound of formula **V**



V

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein

R^1, R^2, R^3, and R^4 are hydrogen or alkyl;

R^5 is halo, hydroxy, amino, or cyano;

R^6 is hydrogen, hydroxy, amino, or cyano;

R^7, R^9, and R^{10} are hydrogen or $-SO_3(R^{12})$;

R^8 and R^{11} are hydrogen; and

R^{12} is an associated cation selected from the group consisting of hydrogen, sodium, or lithium.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1, R^2, R^3, and R^4 are hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is methyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is methyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^4 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is hydroxy. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^5 is amino.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^6 is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^6 is amino.

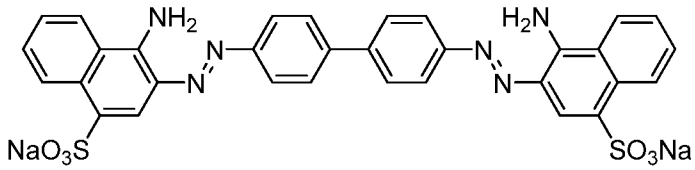
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^7 is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^7 is $-SO_3(R^{12})$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^9 is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^9 is $-SO_3(R^{12})$.

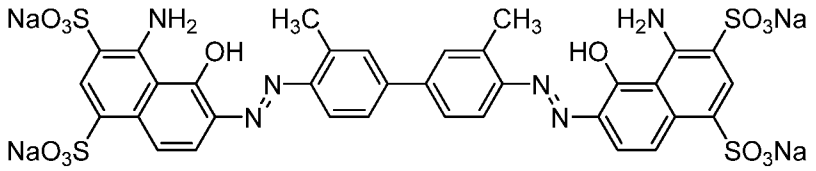
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{10} is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{10} is $-SO_3(R^{12})$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^{12} is sodium.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **V** is not Congo Red, Evan's Blue,



, or



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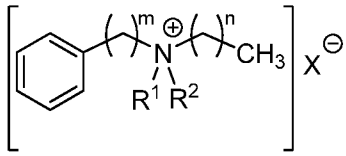
In certain embodiments, the invention relates to a compound of formula **VI**



VI

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

R^1 and R^2 are alkyl;

m is 0, 1, or 2;

n is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19; and

$X^{\ominus}$ is chloride, bromide, or iodide.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is methyl.

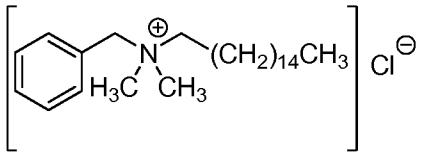
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 and R^2 are the same.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein m is 1.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 13, 14, 15, 16, or 17. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 13, 15, or 17. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 15.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein $X^{\ominus}$ is chloride.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **VI** is not cetalkonium chloride or



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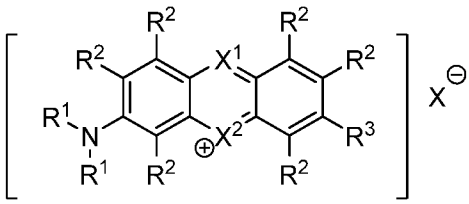
In certain embodiments, the invention relates to a compound of formula **VII**



VII

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

R^1 is hydrogen or alkyl;

R^2 is hydrogen or alkyl;

R^3 is amino or hydroxy;

X^1 is –N= or –C(R^1)=;

X^2 is –S= or –O=;

$X^{\ominus}$ is chloride, bromide, or iodide;

at least one instance of R^1 is alkyl; and

at least one instance of R^2 is alkyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is methyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein both instances of R^1 are alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein both instances of R^1 are methyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein one instance of R^2 is methyl.

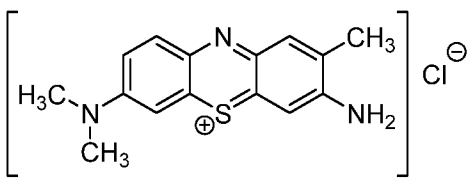
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^3 is amino.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is –N=.

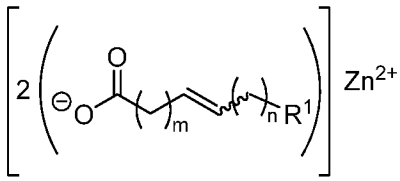
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is –S=.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein $X^{\ominus}$ is chloride.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **VII** is not tolonium chloride or



In certain embodiments, the invention relates to a compound of formula **VIII**



VIII

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14;

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14;

R^1 is hydrogen or methyl; and

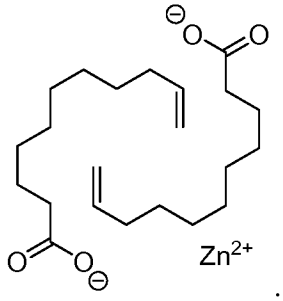
m + n is greater than 4.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein m is 6, 7, 8, 9, or 10. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein m is 6, 8, or 10. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein m is 8.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein n is 0.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **VIII** is not zinc undecylenate or



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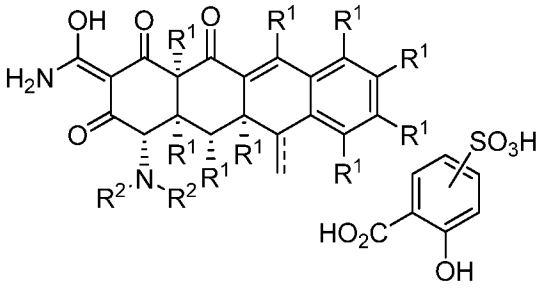
In certain embodiments, the invention relates to a compound of formula **IX**



IX

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

R^1 is hydrogen, halo, hydroxy, alkyl, amino, or cyano;

R^2 is hydrogen or alkyl;

 represents $=CH_2$ or $-CH_3$;

at least two instances of R^1 are hydroxy;

at least one instance of R^1 is hydrogen; and

at least one instance of R^1 is halo.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least three instances of R^1 are hydroxy. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein four instances of R^1 are hydroxy.

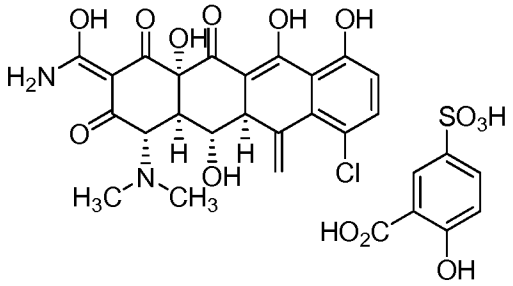
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least two instances of R^1 are hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein at least three instances of R^1 are hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein four instances of R^1 are hydrogen.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein one instance of R^1 is halo. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein one instance of R^1 is chloro.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is alkyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is methyl. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^2 is hydrogen.

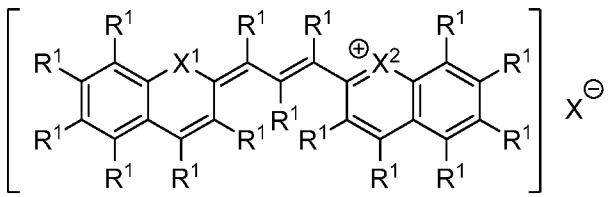
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein  represents $=CH_2$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **IX** is not meclocycline sulfosalicylate salt



or .

In certain embodiments, the invention relates to a compound of formula **X**



X

or a pharmaceutically acceptable salt, solvate, hydrate, prodrug, chemically-protected form, enantiomer or stereoisomer thereof;

wherein, independently for each occurrence,

R^1 is hydrogen or alkyl;

X^1 is $–N(R^1)–$, –S–, or –O–;

X^2 is $=N(R^1)–$, =S–, or =O–;

$X^{\ominus}$ is chloride, bromide, or iodide.

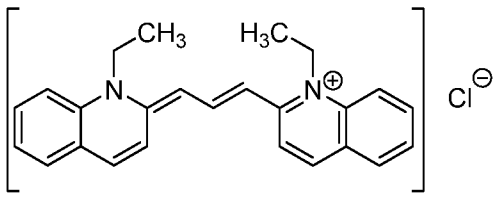
In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is hydrogen. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein R^1 is alkyl.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is $-N(R^1)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is $-N(alkyl)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is $-NH-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is $-N(CH^3)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^1 is $-N(CH^2CH^3)-$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is $=N(R^1)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is $=N(alkyl)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is $=NH-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is $=N(CH^3)-$. In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein X^2 is $=N(CH^2CH^3)-$.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein $X^{\ominus}$ is chloride.

In certain embodiments, the invention relates to any one of the aforementioned compounds, wherein the compound of formula **X** is not quinaldine blue or



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In view of their activity, the compounds of the present invention, either singly or as a mixture, are adaptable to being utilized in various applications of antifungal compositions. In such case, one or more compounds of formulas **I - X**, or a pharmaceutically acceptable salt thereof, may be admixed with a biologically inert carrier, generally with the aid of a surface active dispersing agent, the nature of which would vary depending on whether the use is for the control of pathogens infecting man or animals, or for control of fungi in agriculture, such as in soil or plant parts, or for the control of fungi in inanimate objects.

In compositions for medical applications, the compound may be admixed with a pharmaceutically acceptable carrier, the nature of which will depend on whether the composition is to be topical, parenteral or oral.

If said application is to be topical, the drug may be formulated in conventional creams and ointments, such as white petrolatum, anhydrous lanolin, cetyl alcohol, cold cream, glyceryl monostearate, rose water and the like.

For parenteral applications, the compounds may be formulated in conventional parenteral solutions, such as 0.85 percent sodium chloride or 5 percent dextrose in water, or other pharmaceutically acceptable compositions.

Compositions for oral administration may be prepared by mixing the component drugs with any of the usual pharmaceutical media, including for liquid preparations, liquid carriers, such as water, glycols, oils, alcohols, and the like; and for solid preparations, such as capsules and tablets, solid carriers, such as starches, sugars, kaolin, ethyl cellulose, surface active dispersing agents, generally with lubricants, such as calcium stearate, together with binders, disintegrating agents and the like. Water is the preferred liquid carrier for the compound of the invention.

These compositions are then administered in amounts sufficient to obtain the desired antifungal effect. For medical applications, the method comprises administering to a subject in need of treatment a therapeutically effective antifungal amount of the compounds. The appropriate dose will vary depending on age, severity, body weight and other conditions. For topical application, the compositions are applied directly to the area where control is desired. For internal administration, the composition may be applied by injection or may be administered orally.

For non-medical application, the product of the present invention, either alone or as a mixture, may be employed in compositions in an inert carrier which included finely divided dry or liquid diluents, extenders, fillers, conditioners and excipients, including various clays, diatomaceous earth, talc, and the like or water and various organic liquids, such as lower alkanols, such as ethanol and isopropanol.

Compositions for injection, a preferred route of delivery, may be prepared in unit dosage form in ampules, or in multidose containers. The injectable compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain various formulating agents. Alternatively, the active ingredient may be in powder (lyophillized or non-lyophillized) form for reconstitution at the time of delivery with a suitable vehicle, such as sterile water. In injectable compositions, the carrier is typically comprised of sterile water, saline or another injectable liquid, e.g., peanut oil for

intramuscular injections. Also, various buffering agents, preservatives and the like can be included.

Topical applications may be formulated in carriers, such as hydrophobic or hydrophilic bases to form ointments, creams, lotions, in aqueous, oleaginous or alcoholic liquids to form paints or in dry diluents to form powders.

Oral compositions may take such forms as tablets, capsules, oral suspensions and oral solutions. The oral compositions may utilize carriers, such as conventional formulating agents, and may include sustained release properties as well as rapid delivery forms.

The dosage to be administered depends to a large extent upon the condition and size of the subject being treated, the route and frequency of administration, the sensitivity of the pathogen to the particular compound selected, the virulence of the infection and other factors. Such matters, however, are left to the routine discretion of the physician according to principles of treatment well known in the antibacterial arts. Another factor that influences the precise dosage regimen, apart from the nature of the infection and peculiar identity of the individual being treated, is the molecular weight of the compound.

The compositions for human delivery per unit dosage, whether liquid or solid, may contain from about 0.01% to as high as about 99% of active material, the preferred range being from about 10-60%. The composition will generally contain from about 15 mg to about 2.5 g of the active ingredient; however, in general, it is preferable to employ dosage amounts in the range of from about 250 mg to 1000 mg. In parenteral administration, the unit dosage will typically include the pure compound in sterile water solution or in the form of a soluble powder intended for solution, which can be adjusted to neutral pH and isotonic.

Use of Antifungal Agents

In certain embodiments, the compounds of the invention are useful for the prevention and/or treatment of fungal infections in subjects in need thereof. The compounds of the invention are useful against organisms causing systemic human pathogenic fungal infections, such as, for example, *Cryptococcus neoformans, Aspergillus fumigatus, Aspergillus nidulans, Magnaporthe grisea, Candida albicans, Candida tropicalis, Candida guillermondii, Candida glabrata, Candida pseudotropicalis, Saccharomyces cerevisiae,* and *Aspergillus flavus*. Such systemic human fungal infections

often occur in individuals with a compromised immune system, such as individuals with HIV/AIDS, leukemia, or people who have undergone organ transplantation.

In certain embodiments, the compounds of the instant invention are used in combination with a second antifungal compound. Inhibitors of Sre1 activity or expression, such as the compounds of the invention, act synergistically with azole class antifungal compounds, such as Clotrimazole, Posaconazole, Ravuconazole, Econazole, Ketoconazole and Voriconazole. Thus, in certain embodiment, the compounds of the instant invention are administered in combination with an azole class antifungal agent. In such embodiments, the compound of the invention can be administered before, concurrently or after the azole compound is administered. Certain aspects of the present invention are related to compositions in a pharmaceutically acceptable carrier and their use for the treatment or prevention of fungal infections by administering a therapeutically effective amount of one or both of the compounds.

In certain embodiments, the compounds of the invention are also useful against organisms causing superficial fungal infections. These properties may be effectively utilized by administering compositions containing an antifungal amount of the compound to an area, object or subject, on or in which fungi are to be controlled. Thus, compositions containing an antifungally effective amount of the compound and their use for the control of fungi are aspects of the present invention.

One aspect of the invention relates to a method for treating or preventing a fungal infection in subject, comprising administering to the subject a therapeutically effective amount of a compound of formula **I - X**, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof.

Another aspect of the invention relates to a method for preventing or treating an agricultural fungal infection, comprising administering to the site where growth is to be treated an amount of the compound or composition of a compound of formula **I - X** sufficient to exert an antifungal activity.

In certain embodiments, the present invention relates to any of the aforementioned methods, wherein fungal infection is caused by a fungus of a genus selected from the group consisting of Candida, Aspergillus, Cryptococcus, Mucor, Histoplasma, Blastomyces, Coccidioides, Paracoccidioides, Trichophyton, Epidermophyton, Microsporum, Malassezia, Pseudallescheria, Sporothrix, Rhinosporidium, Fonsecaea, wangiella, Phialophora,

Exophiala, Cladosporium, Alternaria, Aureobasidium, Chaetomium, Curvularia, Drechslera, Mycocentrospora, Phoma, Hendersonula, Scytalidium, Corynespora, Leptosphaeria, Madurella, Neotestudina, Sedosporium, Pyrenochaeta, Geotrichum, Trichosporon, Chrysosporium, Coprinus, Schizophyllum, Pneumocystis, Conidiobolus, Basidiobolus, Paecilomyces, Penicilliun, Acremonium, Fusarium, Scopulariopsis, Saccharomyces, Cephalosporium, Loboa, Rhizopus, Rhizomucor and Absidia.

In certain embodiments, the present invention relates to any of the aforementioned methods, wherein the method of administration of the antifungal compound is selected from the group consisting of oral and parenteral, e.g., i.v. infusion, i.v. bolus and i.m. injection.

In certain embodiments, the present invention relates to any of the aforementioned methods, wherein the administration is by intravenous administration, intramuscular administration or subcutaneous administration.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

EXEMPLIFICATION

Example 1: Sre1 and Stp1 are required Fungal Growth under Hypoxic Conditions

The serotype A reference strain *C. neoformans* H99 contains a single sequence homolog of the human Site-2 protease called *STP1* that is required for hypoxic growth and virulence. *STP1* codes for a 594 amino acid protein that shows a low level of sequence identity to the human Site-2 protease (Fig. 6A). Importantly, the key catalytic residues for Site-2 protease function are conserved, including two histidines and a glutamate in the N-terminus and one aspartate near the C-terminus (Fig. 6A, underlined residues).

To determine whether cells lacking *C. neoformans STP1* produce cleaved Sre1 N-terminus (Sre1N), *stp1*Δ cells were generated by homologous recombination. Using anti-Sre1 antiserum to detect both the full-length form and the cleaved N-terminal form of Sre1, Sre1 cleavage in *stp1*Δ cells was assayed by immunoblot analysis (Fig. 6B). Wild-type and *stp1*Δ cells were grown at ambient oxygen and then switched to 3% oxygen for increasing amounts of time. Unlike wild-type cells where Sre1N (~75 kD) rapidly accumulates under low oxygen, *stp1*Δ cells failed to produce Sre1N (Fig. 6B). In addition, *stp1Δ* cells contained reduced amounts of Sre1 precursor. These data indicate that Stp1 is required for Sre1 proteolysis.

STP1 is additionally required for Sre1 expression. To investigate this further, the levels of *SRE1* mRNA were measured using quantitative PCR (Fig. 6C). Compared to wild-type, *SRE1* transcript levels in *stp1Δ* cells were similar under normoxic conditions but reduced under hypoxic conditions, indicating that under Sre1-activating conditions *STP1* is required for *SRE1* synthesis.

Sre1 is required for normal growth under low oxygen conditions and in the presence of the hypoxia mimetic, cobalt chloride ($CoCl_2$). Serial dilutions of cells were plated on rich medium in the absence or presence of 0.3 mM $CoCl_2$ and grown for 3 days at 30°C (Fig. 6D). In the absence of $CoCl_2$, all strains grew similar to wild-type. However, both *sre1*Δ and *stp1*Δ cells failed to grow on the $CoCl_2$-containing plates. Growth on $CoCl_2$ was rescued by transforming *sre1*Δ and *stp1*Δ with wild-type copies of *SRE1* and *STP1*, respectively.

Example 2: Sre1 and Stp1 are required Virulence *in vivo.*

Virulence studies were performed using Sre1 and Stp1 deletion strains of *C. neoformans* in BALB/c mice. First, it was tested whether the mutant strains displayed growth defects under lab culture conditions. Growth of wild-type, *sre1*Δ and *stp1*Δ strains were monitored in rich medium for 12 hours at 30°C and 37°C (Fig. 7A). Under these conditions, no significant difference in growth between the mutant and wild-type strains was observed. In addition, whether *sre1*Δ and *stp1*Δ cells display significant defects in other known virulence factors, including melanin production and polysaccharide capsule synthesis, was tested. A decrease in melanin production on norepinephrine-containing medium and no difference in capsule size were observed. Next, we infected

Groups of 10 BALB/c mice were infected with wild-type, *sre1*Δ, *stp1*Δ, *sre1*Δ + *SRE1* and *stp1*Δ + *STP1* strains via lateral tail-vein injection and monitored mouse survival over time (Fig. 7B). Mice infected with wild-type serotype A cells succumbed to fatal infection from 7-18 days post-infection. However, cells lacking *SRE1* or *STP1* showed delayed lethality in mice after ~25-40 days post-infection, indicating that Sre1 activation is partially required for normal disease progression in this model of infection. Finally, complementing *sre1*Δ and *stp1*Δ cells with the corresponding wild-type genes restored virulence to these strains.

Example 3: Azole drugs are fungicidal to *sre1Δ* and *stp1Δ* cells

Ergosterol is an essential component of fungal cell membranes. Antifungal therapeutics, including the widely-used azole class of drugs target ergosterol biosynthesis. Studies analyzing both the fungistatic and fungicidal properties of ergosterol biosynthesis inhibitors on wild-type, *sre1*Δ and *stp1*Δ strains were performed. The effects of the antifungal azole drug, itraconazole, and a new sterol biosynthesis inhibitor, 25-thialanosterol were analyzed. 25-thialanosterol is an inhibitor of the fungal-specific sterol biosynthetic enzyme, sterol 24-C-methyltransferase (Erg6). Wild-type, *sre1*Δ and *stp1*Δ cells were grown for 48 hours in rich medium at 30°C in the presence of two-fold serial dilutions of itraconazole or 25-thialanosterol. Low nanomolar concentrations of itraconazole inhibited growth of *sre1*Δ and *stp1*Δ cells, but not wild-type cells (Fig. 8A, left). Importantly, *sre1*Δ and *stp1*Δ strains also displayed growth sensitivity to 25-

thialanosterol (Fig. 8A, right). Given that these two compounds inhibit different ergosterol biosynthetic enzymes, these results indicate that the growth sensitivity phenotype of the two mutants is due to defects in ergosterol biosynthesis and not other non-sterol related effects of azole drugs.

For the treatment of fungal infection, azole antifungals are considered fungistatic but not fungicidal, meaning that the drugs inhibit fungal cell growth but do not actually kill cells. It was examined whether Sre1 activation is required for cell viability under the same culture conditions. The viability of *sre1*Δ and *stp1*Δ strains was dramatically reduced compared to wild-type cells in the presence of both itraconazole and 25-thialanosterol (Fig. 8B). Treating cells with 2.5 nM itraconazole had no effect on wild-type cell viability, but reduced viability of *sre1*Δ and *stp1*Δ cells to 10%. Similarly, treating cells with 2.5 nM 25-thialanosterol had no effect on wild-type cells, but reduced viability of mutant cells to 2%. These data indicated that the decreased growth of *sre1*Δ and *stp1*Δ strains in the presence of these sterol synthesis inhibitors is due, at least in part, to cell-death.

To investigate the kinetics of this killing effect, viability of cells treated for short times was assayed with either 5 nM itraconazole or 2.5 nM 25-thialanosterol, conditions that killed >98% of *sre1*Δ and *stp1*Δ cells after a 48 hour treatment (Fig. 8A). Viability of wild-type cells was not significantly affected by either drug during the 9 hour experiment (Fig. 8C). However, treatment with either itraconazole or 25-thialanosterol rapidly decreased *sre1*Δ and *stp1*Δ cell viability to 37% and 30% after 9 hours for itraconazole and 25-thialanosterol, respectively. This rapid decrease in viability indicates that the Sre1 transcriptional response provides a strong resistance mechanism at early times following exposure to sterol synthesis-inhibiting drugs.

Example 4: Loss of Sre1 Expression Improves Effectiveness of Azole Drugs *in vivo*

As demonstrated above, *C. neoformans* cells lacking the Sre1 pathway activity are hypersensitive to azole anti-fungal drugs and azoles are fungicidal in this setting and cells lacking Sre1 pathway activity display reduced virulence in a mouse model of cryptococcosis. To test whether Sre1 pathway inhibitors function synergistically with azoles in a mouse model of infection, the ability of azole drug treatment to cure cryptococcosis in mice infected with wild-type and *sre1Δ* cells was investigated.

On Day 0, groups of ten mice were infected with either wild-type H99 *C. neoformans* or *sre1*Δ cells (500,000 cells/mouse). Starting on Day 1, infected mice received daily IP injections of either phosphate buffered saline (PBS) or 40 mg/kg of the azole fluconazole. Groups of mice were treated with azole for 14 days and mouse survival was monitored for 140 days.

Mice infected with wild-type cells die by 8 days and mice infected with *sre1Δ* cells showed increased survival, but died within approximately 50 days (Fig. 9). Azole treatment improved survival of mice infected with wild-type cells, but did not cure the infection. However, azole treatment has dramatically increased the survival of mice infected with *sre1Δ* cells, with over 90% of the mice surviving the full 140 days of the experiment (Fig. 9).

Example 5: Generation of a Sre1 Reporter Fungus Strain

The SREBP transcription factor Sre1 responds to low oxygen (hypoxia) to control ergosterol homeostasis in fungi. Sre1 is required in mouse models for productive disease by the human opportunistic fungal pathogens, *Cryptococcus neoformans* and *Aspergillus fumigatus*. As demonstrated above, fungi lacking Sre1 are hypersensitive to anti-fungal drugs that target ergosterol biosynthesis due to an inability to upregulate ergosterol enzymes when sterol synthesis is inhibited. Inhibitors of Sre1 activity are therefore likely to be effective anti-fungal compounds when used either alone or in combination with current and future anti-fungal therapies that inhibit ergosterol biosynthesis. Furthermore, Sre1 is broadly conserved in fungi and thus this anti-fungal approach applies to a broad group of animal and plant fungal pathogens.

To facilitate screening of small molecule inhibitors of Sre1 activity, a *C. neoformans* strain that reports Sre1 as a measure of fluorescent protein expression was generated. In the reporter strain, transcription of a reporter gene coding for a fluorescent protein (GFP) is controlled by the ERG25 promoter. ERG25 codes for an ergosterol biosynthetic enzyme and ERG25 transcription requires Sre1. Expression of GFP by the reporter strain is optimized by expressing the GFP as a fusion protein that contains the first 30 amino acids of Erg25p (Fig. 1). The reporter strain also contains a red fluorescent protein (DsRed) expressed from a constitutive promoter. Inhibitors of Sre1 activity will lower the GFP/DsRed signal ratio.

The Sre1 reporter strain was generated as follows. First, to generate a strain containing stably-integrated pCB61-1 (Fig. 2 and 4), 1 μg of plasmid pCB61-1 was linearized by digestion with XmnI restriction enzyme. Linearized plasmid was ethanol precipitated and resuspended in 5 μl water. Wild type *C. neoformans* H99 cells were transformed with pCB61-1 by electroporation. Transformed cells were plated on the rich medium, YES (0.5%(w/v) yeast extract plus 3%(w/v) glucose and supplements, 225 μg/ml each of uracil, adenine, leucine, histidine, and lysine), and grown overnight at 30 degrees. Transformants were then selected by replica-plating onto YES plates containing 150 μg/ml hygromycin B (Roche). Colonies were picked and passaged three times on YES without hygromycin and then replated on YES + 150 μg/ml hygromycin to test for stable integration of the plasmid into the chromosomal DNA.

A stable transformant with high levels of pH3-DsRED expression was selected for generating the reporter strain. Plasmid pCB54-1 (Fig. 3 and 5) was linearized with I-SceI restriction enzyme to expose telomeric ends which allowed the plasmid to be maintained episomally. Linearized plasmid was ethanol precipitated, resuspended in 5 μl water and transformed into the pCB61-1 containing *C. neoformans* H99 cells by electroporation. Transformed cells were plated on the rich medium, YES, and grown overnight at 30 degrees. Transformants were then selected by replica-plating cells onto YES plates containing 100 μg/ml G418 (Invitrogen). Transformants were further passaged on YES+G418.

Example 6: Screening a Small Molecule Library Using a Sre1 Reporter Fungus Strain

The *C. neoformans* reporter strain described above was used to screen for compounds that lowered expression of an Sre1-dependent reporter gene (GFP, operably linked to the ERG25 promoter), but that had no effect on a control reporter gene (DsRED, operably linked to the Histone H3 promoter).

To identify inhibitors of Sre1 activity or expression, 3131 compounds were screened in a 96-well plate format. The screened library included 1,754 US-FDA approved drugs and others in various phases of development. Compounds were screened at 10 μM. Initial screening yielded 108 hits. Only 45 were available for retesting, and which yielded 11 clear inhibitors of Sre1 reporter gene expression. (Table 2).

Table 2: Inhibitors of Sre1 Activity or Expression

Inhibitor	Original plate #	Drug name	Indication	Description
1	15B10	Dichlorophen (2,2 methylene bis 4-chlorophenol)	Anthelmintic (Cestodes)	used to treat tape worm infection
2	18F2	Ursolic acid		
3	19G4	Pentifylline	Vasodilator	purine analog similar to caffeine
4	22H3	Benzbromarone	MISC- Uricosuric	inhibits CYP2C9. related to amiodarone. used to treat gout to increase excretion of uric acid in urine (and lowering blood levels of uric acid)
5	25F5	Congo red	Diagnostic aid (amyloidosis)	pH indicator
6	28G10	Cetalkonium chloride (Benzyldimethylhexadecylammonium chloride)	Antibacterial (topical)	cause dissociation of cellular membrane lipid bilayers
7	30G5	Tolonium chloride (Toluidine blue O)	Hemostatic	dye for lignin
8	31B2	Zinc undecylenate	Antifungal (topical)	used against fungal skin infections such as athlete's foot, ringworm, jock itch, Candida albicans
9	33D7	Meclocycline sulfosalicylate salt	Antibiotic	tetracycline antibiotic. Inhibits the binding of aminoacyl-tRNA to the mRNA-ribosome complex
10	34C4	Quinaldine blue (Pinacyanol chloride)	Antineoplastic	
11	37G2	Evans blue	Diagnostic Aid (blood volume determination)	binds serum albumin

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed.

INCORPORATION BY REFERENCE

All of the U.S. patents and U.S. patent application publications cited herein are hereby incorporated by reference.

We claim:

1. An isolated nucleic acid molecule comprising a sequence encoding a reporter protein operably linked to the promoter of a gene that is upregulated by Sre1.

2. The isolated nucleic acid molecule of claim 1, wherein the promoter is an ERG25 promoter.

3. The isolated nucleic acid molecule of claim 2, wherein the reporter protein is a fusion protein that comprises amino acids 1-10 of Erg25p.

4. The isolated nucleic acid molecule of claim 2, wherein the reporter protein is a fusion protein that comprises amino acids 1-20 of Erg25p.

5. The isolated nucleic acid molecule of claim 2, wherein the reporter protein is a fusion protein that comprises amino acids 1-30 of Erg25p.

6. The isolated nucleic acid molecule of claim 2, wherein the reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

7. A plasmid comprising a first nucleic acid sequence encoding a first reporter protein operably linked to the promoter of a gene that is upregulated by Sre1.

8. The plasmid of claim 7, wherein the promoter is an ERG25 promoter.

9. The plasmid of claim 8, wherein the first reporter protein is a fusion protein that comprises amino acids 1-10 of Erg25p.

10. The plasmid of claim 8, wherein the first reporter protein is a fusion protein that comprises amino acids 1-20 of Erg25p.

11. The plasmid of claim 8, wherein the first reporter protein is a fusion protein that comprises amino acids 1-30 of Erg25p.

12. The plasmid of claim 8, wherein the first reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

13. The plasmid of claim 8, further comprising a second nucleic acid sequence encoding a second reporter protein operably linked to a second promoter.

14. The plasmid of claim 13, wherein the second promoter is a constitutive promoter.

15. The plasmid of claim 13, wherein the second promoter is the Histone H3 promoter.

16. The plasmid of claim 13, wherein the second reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

17. A fungal cell comprising a first nucleic acid sequence encoding a first reporter protein operably linked to the promoter of a gene that is upregulated by Sre1.

18. The fungal cell of claim 17, wherein the promoter is an ERG25 promoter.

19. The fungal cell of claim 18, wherein the first nucleic acid sequence is integrated into the chromosomal DNA of the fungal cell.

20. The fungal cell of claim 18, wherein the first nucleic acid sequence is extra-chromosomal.

21. The fungal cell of claim 18, wherein the first reporter protein is a fusion protein that comprises amino acids 1-10 of Erg25p.

22. The fungal cell of claim 18, wherein the first reporter protein is a fusion protein that comprises amino acids 1-20 of Erg25p.

23. The fungal cell of claim 18, wherein the first reporter protein is a fusion protein that comprises amino acids 1-30 of ERG25p.

24. The fungal cell of claim 18, wherein the first reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

25. The fungal cell of claim 18, wherein the fungal cell is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans.*

26. The fungal cell of claim 18, further comprising a second nucleic acid sequence encoding a second reporter protein operably linked to a second promoter.

27. The fungal cell of claim 26, wherein the second nucleic acid sequence is integrated into the chromosomal DNA of the fungal cell.

28. The fungal cell of claim 26, wherein the second promoter is a constitutive promoter.

29. The fungal cell of claim 26, wherein the second promoter is the Histone H3 promoter.

30. The fungal cell of claim 26, wherein the fungal cell is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans.*

31. The method of claim 26, wherein the second reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker or a counter-selectable marker.

32. A method of determining whether an agent is an inhibitor of Sre1 expression or activity comprising:

a) contacting a fungal cell with the agent, wherein the fungal cell comprises a first nucleic acid sequence encoding a first reporter protein operably linked to an ERG25 promoter and a second nucleic acid sequence encoding a second reporter protein operably linked to a constitutive promoter; and

b) determining the expression level of the first reporter protein compared to the second reporter protein;

wherein a reduction of the level of the first reporter protein compared to the second reporter protein indicates that the agent is an inhibitor of Sre1 expression or activity.

33. The method of claim 32, wherein the first nucleic acid sequence and the second nucleic acid sequence are integrated into the chromosomal DNA of the fungal cell.

34. The method of claim 32, wherein the first reporter protein is a fusion protein that comprises amino acids 1-10 of Erg25p.

35. The method of claim 32, wherein the first reporter protein is a fusion protein that comprises amino acids 1-20 of Erg25p.

36. The method of claim 32, wherein the first reporter protein is a fusion protein that comprises amino acids 1-30 of Erg25p.

37. The method of claim 32, wherein the first reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

38. The method of claim 32, wherein the fungal cell is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*.

39. The method of claim 32, wherein the second promoter is a constitutive promoter.

40. The fungal cell of claim 39, wherein the second promoter is the Histone H3 promoter.

41. The method of claim 39, wherein the second reporter protein is selected from the group consisting of: a fluorescent protein, a luciferase protein, a selectable marker and a counter-selectable marker.

42. A method of treating or preventing an infection by a fungus in a subject in need thereof comprising administering to said subject a compound selected from the group consisting of: Dichlorophen, Ursolic acid, Pentifylline, Benzbromarone, Congo Red, Cetalkonium Chloride, Tolonium Chloride, Zinc Undecylenate, Meclocycline Sulfosalicylate Salt, Quinaldine Blue, Evan's Blue, pharmaceutically acceptable salts thereof and derivatives thereof.

43. The method of claim 42, wherein the fungus is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*.

44. The method of claim 42, further comprising administering a second antifungal drug.

45. The method of claim 44, wherein the second antifungal drug is an azole drug.

46. A method reducing ERG25 expression in a fungus comprising contacting the fungus with a compound selected from the group consisting of: Dichlorophen, Ursolic acid, Pentifylline, Benzbromarone, Congo Red, Cetalkonium Chloride, Tolonium Chloride, Zinc Undecylenate, Meclocycline Sulfosalicylate Salt, Quinaldine Blue, Evan's Blue, a pharmaceutically acceptable salt thereof and a derivative thereof.

47. The method of claim 46, wherein the fungus is *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans, Magnaporthe grisea* or *Candida albicans*.

Figure 1

ERG25 Promoter Sequence:

TAGGATCCCTGTCAATGTGCAGTCAACTCACGGACTGACTACAGTACTTCAATGAATGCCGTAAAAAGAAGTG
AAGGCTTTTTACCTTCCAACATGATGGTTGCAATACGATTGCTATAGCCATGACCTAAACATCTGCAGAAGAG
GTCAACGCATTTTGTTGACGCTTCTATTGGCTTGAACGGGGAGCAAGCGGCTCGTGCACCATCATCTGCTTGG
GCTAAGAAACGCATCTGAACTATCGAATCGTTTACCTGCCCAGTGGTCAGTGACTACCTCTATAACTTTCCTT
CCATCTTTGAGTGCATATGATGTTCGTTTCATAACAGTGTGCAAACGACGCGACCCCTGCGCTGGCCGCTGAT
CGGTCAAAAGTCCATGCTCAGTAATGTAAATCTATCCTTACAACATGGTAAATACACTTAATGCCCATCACCT
CATGTCTATAACGCTCTGCGAATTGCATTAAACAGGCTTGGGAAGCTTGTTGACTGTCTCGAGATTATATAGC
ACATTACGCGAATAGTTGGAGAAGAGTTGAGACGTGATGTGAATGGCTCCAGCAGAGTCAAGGTATTCCTTGC
GAGTCGCGTCAGCTTAACGTGTCATACGACAAACTTTTGACCATGGCGCCAAGGCAGGGCCAGTAAACATACT
TTGAGGCAGTGATTTAATTCGATAATCGCTTCGTTTGACTTGTCCTAGACGAAGCAGTCAGCGGAAGTATGGA
TGCATCTTCCGAATGGTGCCAACGTACGACATTTTTTATAATTCGCTCTAGCAGTACAGCCTGCTCTGCTTCG
TGTGGGTTTTCCATTGCTTTCGTATAAATTCGGAAGAGGAATGGCGTTTGTTGTTGTTTATAGGAGCTCGTTA
AGGGGTGCGGAACGAAATGCGGAGATATTTTGAGAGTCCGAGGATCGTACCACTGTTGTTTTTGCTGGAATTT
GCCGCGTCGTCGCCTGGCGTCGTTCCACTTTTTTTTTTTT

ERG25aa1-30 – CnGFP Sequence

ATGGCCGCTGCAGCCGCCTTTGACCTGCTCGACAAGTACATCCCCGGCGCCTCAGATTCTCTGACCATTGTCA
ATGCCACCACCGCGCAAATGTCCAAGGGTGAGGAGCTCTTCACCGGTGTCGTCCCCATCCTCGTCGAGCTCGA
CGGTGACGTCAACGGTCACAAGTTCTCCGTCTCCGGTGAGGGTGAGGGTGACGCCACCTACGGTAAGCTCACC
CTCAAGTTCATCTGTACCACCGGTAAGCTCCCCGTCCCCTGGCCCACCCTCGTCACCACCCTCACCTACGGTG
TCCAGTGTTTCTCCCGATACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAGGGTTA
CGTCCAGGAGCGAACCATCTTCTTCAAGGACGACGGTAACTACAAGACCCGAGCCGAGGTCAAGTTCGAGGGT
GACACCCTCGTCAACCGAATCGAGCTCAAGGGTATCGACTTCAAGGAGGACGGTAACATCCTCGGTCACAAGC
TCGAGTACAACTACAACTCCCACAACGTCTACATCATGGCCGACAAGCAGAAGAACGGTATCAAGGTCAACTT
CAAGATCCGACACAACATCGAGGACGGTTCCGTCCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGT
GACGGTCCCGTCCTCCTCCCCGACAACCACTACCTCTCCACCCAGTCCGCCCTCTCCAAGGACCCCAACGAGA
AGCGAGACCACATGGTCCTCCTCGAGTTCGTCACCGCCGCCGGTATCACCCACGGTATGGACGAGCTCTACAA
GGCCGCCGCCGCCGCCGCCGCCGCCGAGGAGCAGAAGCTCATCTCCGAGGAGGACCTCTAAGGATCCGGCCGC
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Figure 2

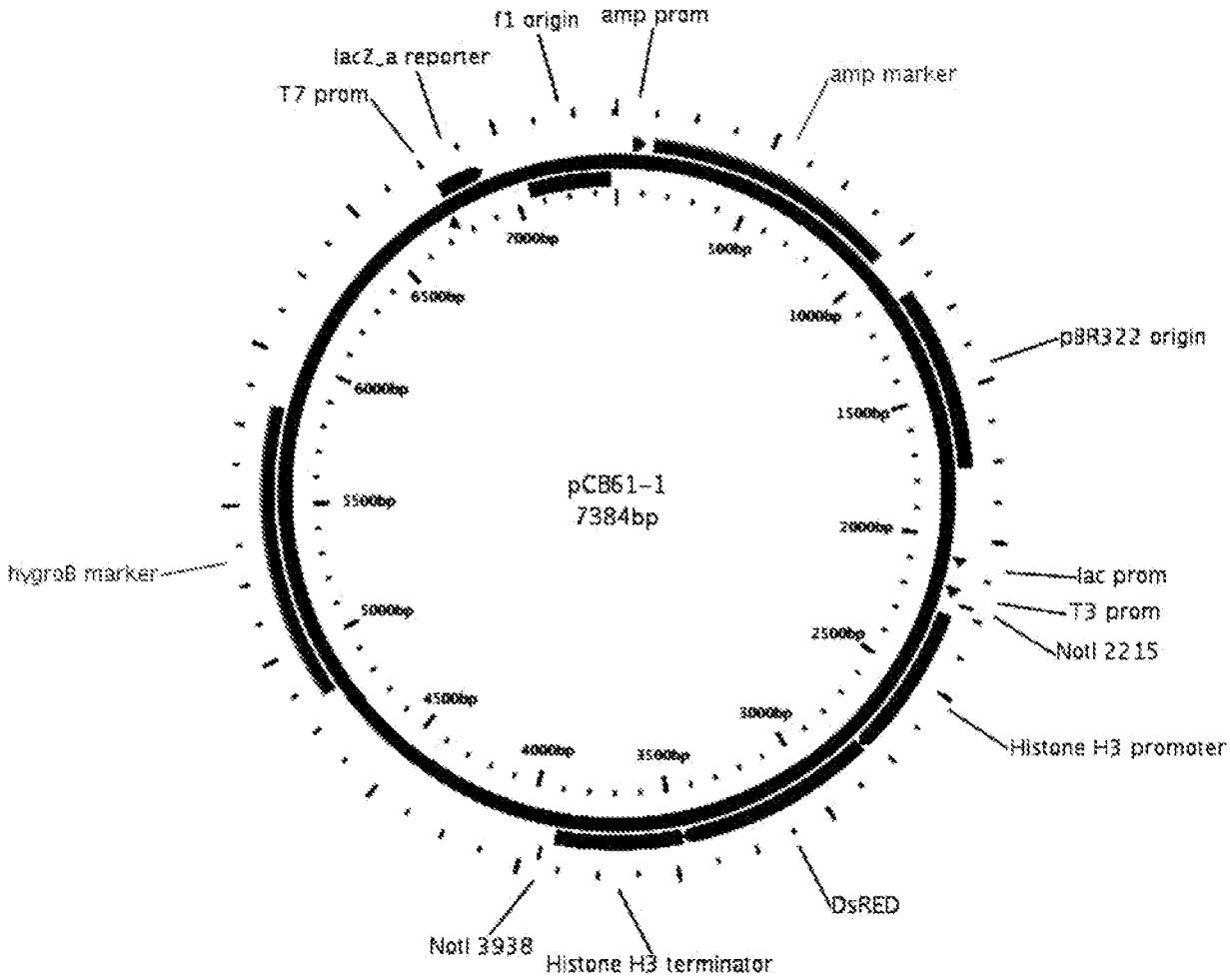
f1 origin
amp prom
lacZ_a reporter
amp marker
T7 prom
pBR322 origin
pCB61-1
7384bp
hygroB marker
lac prom
T3 prom
NotI 2215
Histone H3 promoter
DsRED
NotI 3938
Histone H3 terminator

Figure 3

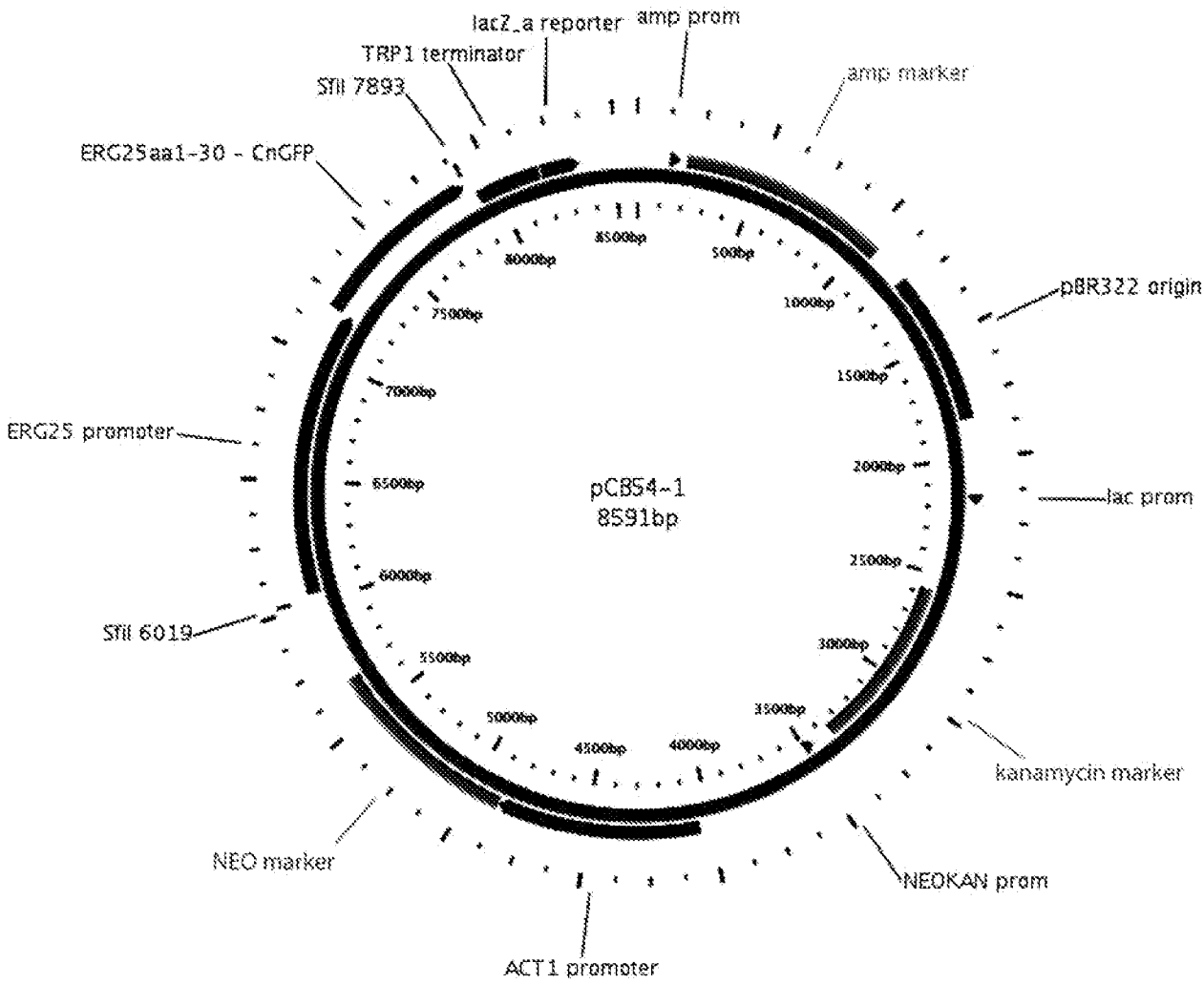
lacZ_a reporter
amp prom
TRP1 terminator
amp marker
STII 7893
ERG25aa1-30 - CnGFP
pBR322 origin
ERG25 promoter
lac prom
pCB54-1
8591bp
STII 6019
kanamycin marker
NEO marker
NEOKAN prom
ACT1 promoter

Figure 4

pCB61-1 Sequence, 7384 bp

GCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCT
CATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGT
GTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAA
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GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCC
CGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCAC
CAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGA
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CTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTT
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TGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGC
AGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTC
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TAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAAC
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GTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC
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GAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGTCCATCTACATGGCCAAGAAGCCCGTGCAGCTG
CCCGGCTACTACTACGTGGACTCCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGT
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CGTGTATAGAAAGAACAATCAAGCGTACAAGAGCTCGATATCTTAATTAAGAATTCGCCCTTAGGCTGGCGGC
CGCTCTAGAACTAGTGGATCACTCGCTATTGTCCAGGCTGCGAGGATGTGAGCTGGAGAGCGGGGCACGGGAA
GGGGAGGACTCACATAAGCATGCAGGATTCGAGTGGCATGGTGTGCGCTGAGTGTATGGTTGTCGGAGGAGAG
GATGATGGTAACAACAATAGCAGCAACGTCACTCGACGCGCGTCCGGTGTGCCACACGGGGTAACGCCGAGTC

Figure 4 (Continued)

GCCGTCAGGGTCGCCGAGACCACTCTCACAGCGTCACCGTTGGCACCAGCTCAGCTTACAGCTTCTATCCTCC
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TCCCTTGCCCGCCACTCAGTCCTCCATCCTCCACTAATCCACCTTATCGCACCCACCGCCTATCGCACATCCG
AGCACAATGCTGGGCCTGCCAGGGGCTGCTAGATGGTGCTCTCCCCACGCTGATCTGCATGCCGGCCATTGGA
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GGATTGACGGTCGCCGAGAGGACGACGTGGCGTTCGACAACGAGGGCCGATAGCACCGCATCGCCTCGACCTG
CATCCATCTGCCTTGTCCTTTTGGTGGAACAATCCATCCGTGCTGGTGCCACACGCATAGCTGGAAGAGATGG
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TCTCCTAATATGCAGCCTCTTTGCTAATTGTCTTTTTCCATTAGTAAACTCGCCCAACATGTCTATGGAAGAA
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CGCGTCAGGTG

Figure 5

pCB54-1 Sequence, 8591 bp

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ACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGT
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CACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCC
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CCGGCGCGATCTGCAGAATTCGCCCTTGCTGCGAGGATGTGAGCTGGAGAGCGGCGCACGGGAAGGGGAGGAC
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Figure 5 (Continued)

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CTCACGGACTGACTACAGTACTTCAATGAATGCCGTAAAAAGAAGTGAAGGCTTTTTACCTTCCAACATGATG
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TTTCATAACAGTGTGCAAACGACGCGACCCCTGCGCTGGCCGCTGATCGGTCAAAAGTCCATGCTCAGTAATG
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CGACAAACTTTTGACCATGGCGCCAAGGCAGGGCCAGTAAACATACTTTGAGGCAGTGATTTAATTCGATAAT
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CCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATT
TCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGC
CAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTC
CGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA

Figure 6

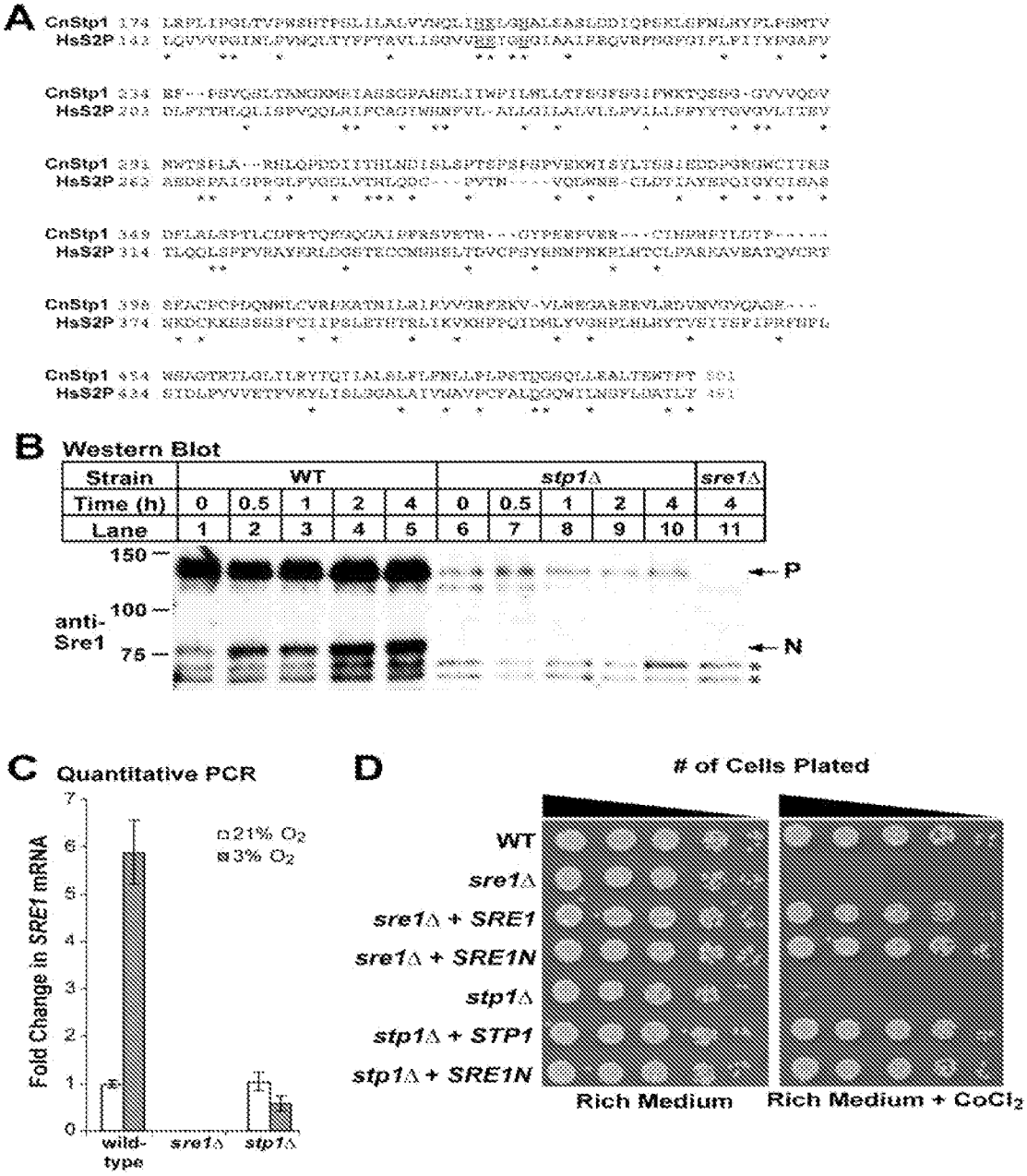

A
CnStp1
HsS2P
B
Western Blot
Strain
WT
stp1Δ
sre1Δ
Time (h)
0
0.5
1
2
4
Lane
1
2
3
4
5
6
7
8
9
10
11
150
100
75
anti-Sre1
P
N
C
Quantitative PCR
21% O2
3% O2
Fold Change in SRE1 mRNA
wild-type
sre1Δ
stp1Δ
D
# of Cells Plated
WT
sre1Δ
sre1Δ + SRE1
sre1Δ + SRE1N
stp1Δ
stp1Δ + STP1
stp1Δ + SRE1N
Rich Medium
Rich Medium + CoCl2

Figure 7

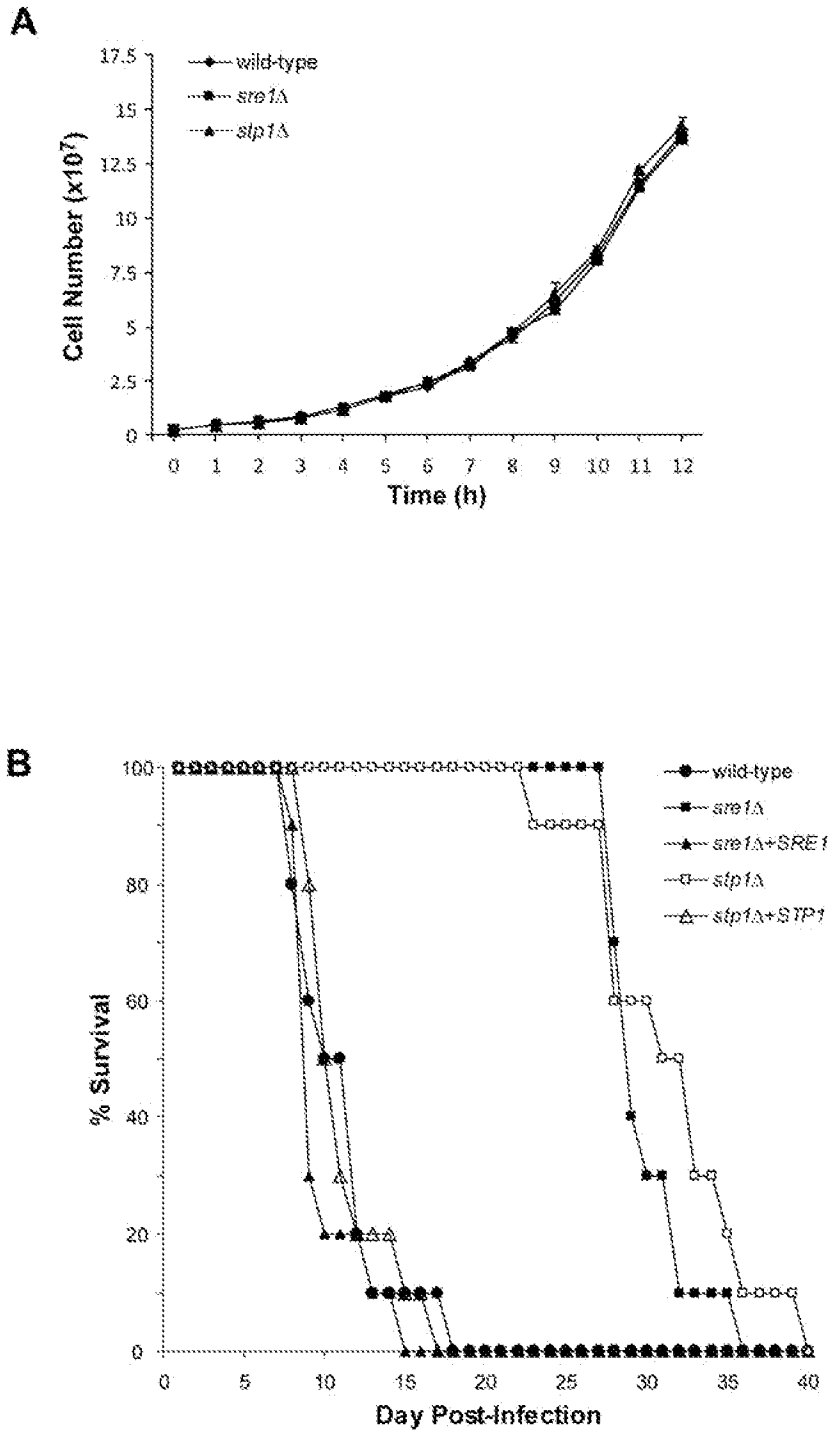

A
wild-type
sre1Δ
stp1Δ
Cell Number (x10^7)
Time (h)
B
wild-type
sre1Δ
sre1Δ+SRE1
stp1Δ
stp1Δ+STP1
% Survival
Day Post-Infection

Figure 8

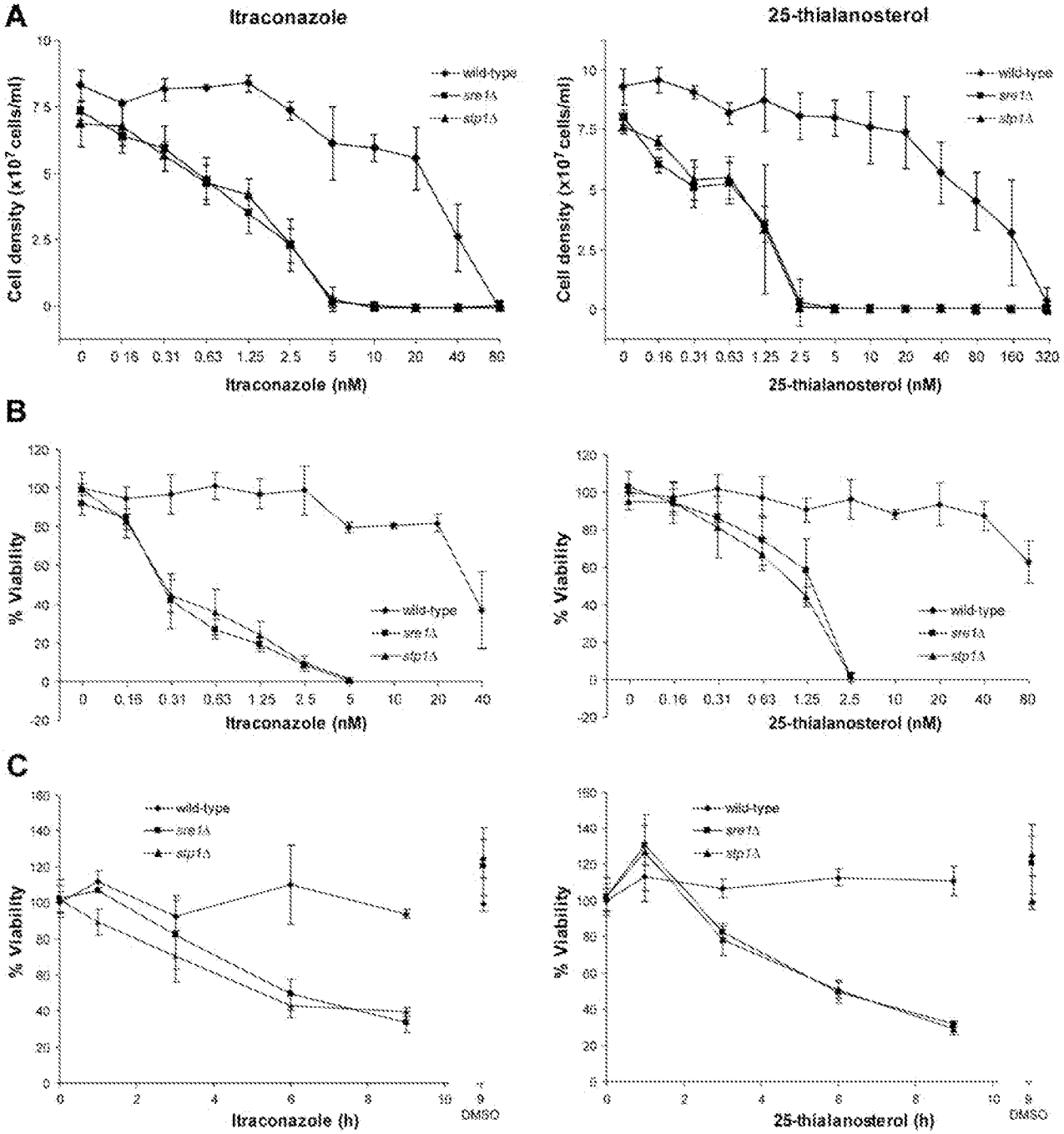
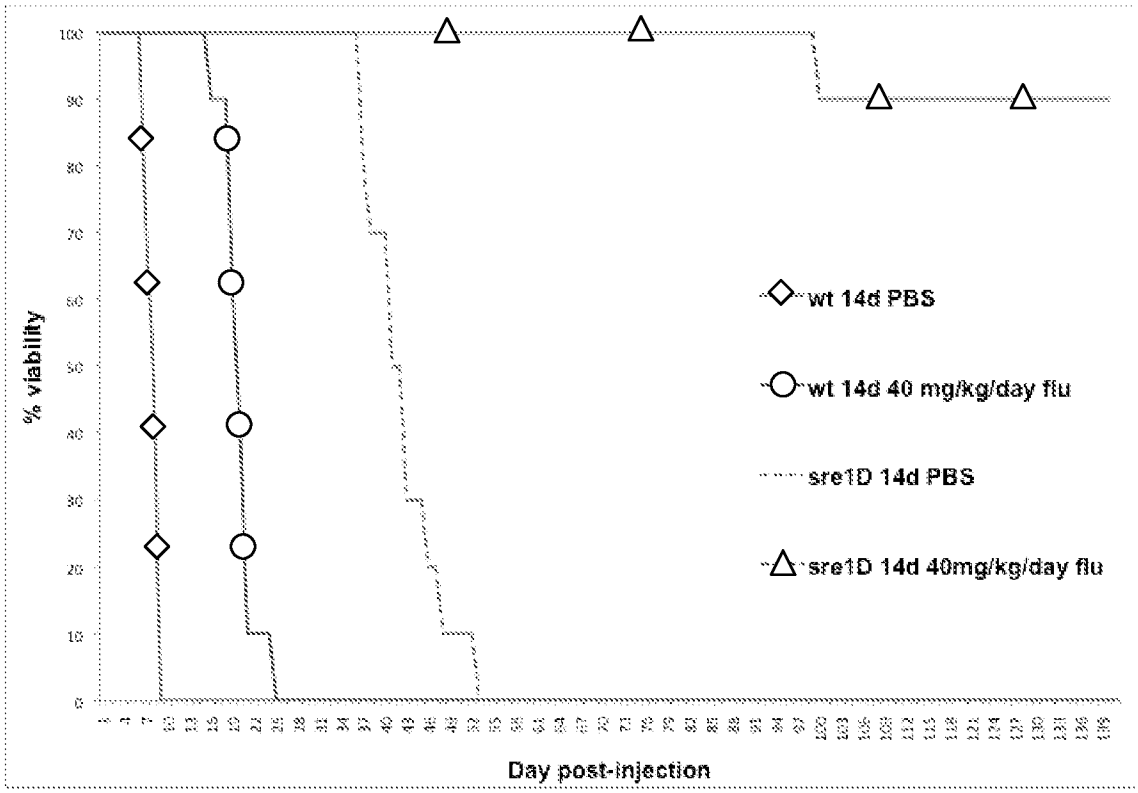

A
Itraconazole
25-thialanosterol
Cell density (x10^7 cells/ml)
wild-type
sre1Δ
stp1Δ
Itraconazole (nM)
25-thialanosterol (nM)
B
% Viability
Itraconazole (nM)
25-thialanosterol (nM)
C
% Viability
Itraconazole (h)
25-thialanosterol (h)
DMSO

Figure 9


% viability
Day post-injection
wt 14d PBS
wt 14d 40 mg/kg/day flu
sre1D 14d PBS
sre1D 14d 40mg/kg/day flu