

MODIFIED PROTEINS

SEQUENCE LISTING

5 The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on August 7, 2023, is named 70384US01P_SL.xml and is 94,459 bytes in size.

FIELD OF THE INVENTION

10 The present invention relates to the field of modified proteins, immunogenic compositions and vaccines comprising the modified proteins, their manufacture and the use of such compositions in medicine. More particularly, it relates to a modified Als3 (Agglutinin-like sequence 3 of *Candida albicans*) protein. The modified Als3 protein can be used as a carrier protein for other antigens, particularly saccharide antigens or other antigens lacking T cell epitopes.

BACKGROUND TO THE INVENTION

15 The impact of fungal infections on humans is a serious public health issue that has received much less attention than bacterial infection and treatment, despite ever-increasing incidence exacerbated by an increased incidence of immunocompromised individuals in the population. Fungal infections are also detrimental to the well-being of grazing livestock, with milk production in dairy
20 cows, and body and coat condition adversely affected by fungal infections. *Candida*, *Cryptococcus*, *Aspergillus*, and *Pneumocystis* are the most common fungal genera causing invasive human infections. *Candida* species, in particular, cause some of the most prevalent fungal infections and is the leading fungal pathogen worldwide. *Candida* species are early colonizers acquired at or near human birth primarily by physical contact. These organisms are able to colonize the skin, as well as the
25 gastrointestinal, and reproductive tracts of humans [9,10]. However, in certain situations (*e.g.* situations of immunosuppression), these organisms can become pathogenic and can cause a broad spectrum of human infections. During the last decades, the incidence of human infections caused by *Candida* genus has increased significantly (Sobel, 2007; Pfuller, 2011). *Candida* infections can be superficial or invasive. Invasive candidiasis are difficult to treat. Globally, an estimated 700,000
30 persons a year suffer from invasive candidiasis, with an associated mortality that may exceed 50%. Furthermore, *Candida* can also cause mucocutaneous infections, such as vulvovaginal candidiasis which, while rarely lethal, are associated with significant morbidity. Candidiasis have an estimated economical burden of \$3 billion in medical costs.

35 *Candida albicans* is the most studied member of the genus and is the most common opportunistic pathogen and cause of invasive fungal infection in hospitalized human patients (Sobel, 2007; Pfuller, 2011). *C. albicans* is a highly adaptable fungal species that is prevalent in nosocomial

infections, and immunocompromised individuals are particularly at risk. *C. albicans* has a large repertoire of virulence factors that allows its transition from commensal organism (yeast form) to pathogen (hyphal form). For example, *C. albicans* is a commensal in the vaginal epithelium. Certain environmental conditions trigger the morphogenesis of *C. albicans* from the commensal yeast form to the pathogenic hyphal form. The hyphal form starts entering the lumen and breaches mucosal barriers, which leads to the symptomatic infection, e.g., vulvovaginal candidiasis (VVC) in women. Worldwide, approximately 70% - 80% of all women will be affected at least once in their lifetime by VVC, with *C. albicans* causing >85% of these infections. Up to 10% of these women will suffer from recurrence. Recurrent VVC or RVVC (defined as > 3 episodes/year) is more serious and refractory to therapy. RVVC results in reduced quality of life, a strong negative impact on work and social life, as well as increased associated healthcare costs. By 2030, the population of women with RVVC is estimated to increase to 158 million.

Despite the high mortality and morbidity rate of invasive fungal infections (IFIs), treatment options are limited and the anti-fungal drug discovery pipeline is under developed (Perfect, 2017). In addition, antifungal therapies are often ineffective. Moreover, the widespread use of antifungals in agriculture and clinical settings has led to the emergence of multidrug-resistant fungal infections (e.g. multidrug resistant *Candida auris* and other non-*albicans* species), which represent major threats to food security and human health (Nguyen et al., 2021). In addition, despite the substantial global burden of human fungal infections, there are no licensed vaccine currently available to prevent and/or control human fungal infections (Oliviera et al., 2021). Reasons for the lack of clinically available fungal vaccines include the very high costs related to manufacturing of the antigens and toxicology issues. In addition, fungal vaccines composed of whole organism (live-attenuated or killed fungal cells) show high immunogenicity, which makes them most likely to generate protective response but there is a potential risk of infection. Therefore, there is a clear and unmet need to identify novel targets and develop new classes of anti-fungal agents, including vaccines against pathogenic fungi (e.g., *C. albicans*) that can safely be produced in high quantities.

Given their theoretical safety advantages, identification of protective antigens for use in subunit vaccines has been the focus of much research. A major difference between the Fungi and Animalia kingdoms is the presence of cell wall on almost all fungal cells. The complex fungal cell wall is vital for maintaining cellular shape and integrity (Garcia-Rubio et al., 2020). An analysis of cell wall composition across a range of *Candida* species, including *C. albicans*, shows that the inner cell wall is composed mainly of β -glucans (β -1,3 and β -1,6 linked polymers of glucose), while the outer cell wall is comprised of highly glycosylated cell wall proteins that are decorated with N- and O-linked terminal mannans (branched polymer of mannose linked via α -1,2, α -1,3, α -1,4, α -1,6 and β -1,2 glycosidic bonds) (Ahmadipour et al., 2021, The Cell Surface, 7:100063). Consequently, these polysaccharide

components of the fungal cell wall, β -glucans and mannans, could be interesting candidates for developing safe and efficacious subunit fungal vaccines. However, polysaccharides are T-independent antigens that elicit antibody production via B lymphocytes without involvement of T-cells. Polysaccharides may elicit a long-lasting T-cell-dependent immune response in humans if they are coupled to a protein carrier that contains T-cell epitopes. Indeed, conjugation of T-independent antigens to carrier proteins has been established as a way of enabling T-cell help to become part of the immune response for a normally T-independent antigen. In this way, an immune response can be enhanced by allowing the development of immune memory and boost stability of the response. Successful conjugate vaccines against prokaryotic pathogenic bacteria have been developed by conjugating bacterial capsular saccharides to carrier proteins, the carrier protein having the known effect of turning the T-independent saccharide antigen into a T-dependent antigen capable of triggering an immune memory response. However, to date, it has not been possible to develop a successful conjugate vaccine against eukaryotic pathogenic fungi such as *Candida* by conjugating fungal cell wall polysaccharides to fungal carrier proteins. The present invention provides, for the first time, a new class of eukaryotic *Candida* glycoconjugate vaccines produced using the process of bioconjugation in a prokaryotic bacteria, namely *Escherichia coli*.

SUMMARY OF THE INVENTION

A glycoconjugate is a hybrid molecule composed of a carrier protein and multiple polysaccharide chains, wherein the polysaccharides are covalently linked to the carrier protein. This linkage of the antigenic polysaccharide to a carrier protein has brought significant advances in the field of vaccinology, eliciting a T-cell-dependent response characterized by the induction of immunological memory and improved immunogenicity. The standard approach to production of glycoconjugate vaccines is a chemical conjugation process that necessitates long development timelines, as the process requires extensive optimization for each individual target antigen. Additionally, the complexity of the production process results in high costs for such products. In contrast, bioconjugation is an innovative technology that allows the production of glycoconjugate vaccines in a biological environment (e.g., *E. coli*) to preserve native immunogenic structures. Using recombinant DNA technologies, the *E. coli* glycan biosynthesis machinery is genetically modified to produce the target polysaccharide antigen and covalently link it to an asparagine residue in a consensus sequence on a carrier protein. Thus, the glycoconjugate vaccine is produced entirely in *E. coli* in a single-step process, resulting in advantages for process reproducibility and robustness, while decreasing manufacturing cost. Using this bioconjugation technology, several glycoconjugate vaccines against prokaryotic organisms such as Gram negative bacteria (e.g. *Shigella*) and Gram positive bacteria (e.g. *Staphylococcus aureus* or *S. pneumoniae*) have been developed and tested in clinical trials. However, because *Candida* is a eukaryote, it has not been straightforward to transfer the biochemical pathway required for bioconjugation and production of *Candida* glycoproteins into *E.*

coli (as was previously done for bacterial lipopolysaccharides or capsular saccharides). Consequently, due to the higher complexity of the *Candida* bioconjugation process, a *Candida* glycoconjugate vaccine in *E. coli* has not previously been successfully produced.

5 The present invention provides modified Agglutinin-like sequence 3 (Als3) proteins from *C. albicans* comprising at least one consensus sequence for glycosylation (*e.g.* D/E-X-N-Z-S/T) for use in conjugation to an antigen (*e.g.* *Candida* polysaccharide). The present invention further provides a glycoconjugate comprising the modified Als3 carrier protein linked to the *Candida* polysaccharide antigen at one or more asparagine residues on the modified Als3 protein, as well as methods of producing the *Candida* glycoconjugate vaccine in a host cell (*e.g.*, *E. coli*). The present invention
10 further provides methods of preventing and/or treating RVVC using the *Candida* glycoconjugate vaccine.

Accordingly, there is provided in certain aspects of the present invention, a modified Agglutinin-like sequence 3 (Als3) protein comprising amino acid residues 18-316 of amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%,
15 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, modified in that the amino acid sequence comprises one or more consensus sequences comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

According to further aspects of the invention, there is provided a modified Als3 protein of the invention, wherein the modified Als3 protein further comprises at least one Fructose biphosphate
20 aldolase-1 (Fba) peptide comprising an amino acid sequence of YGKDVKDLFDYAE (SEQ ID NO: 3) or an amino acid sequence at least 70%, 80%, 85%, 90%, or 92% identical to SEQ ID NO: 3.

According to further aspects of the invention, there is provided a modified Als3 protein of the invention comprising an amino acid sequence of SEQ ID NO: 10.

According to further aspects of the invention, there is provided a modified Als3 protein of the
25 invention comprising an amino acid sequence of SEQ ID NO: 11.

According to further aspects of the invention, there is provided a conjugate (*e.g.* bioconjugate) comprising a modified Als3 protein of the invention and at least one saccharide antigen.

According to further aspects of the invention, there is provided a modified Als3 protein of *Candida albicans* consisting of: (1) an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11; and
30 (2) at least one saccharide antigen of *Candida*, wherein the at least one saccharide antigen is a β -1,3 glucan polymer consisting of at least six consecutive β -1,3 linked glucose molecules, and wherein the at least one saccharide antigen is linked to at least one of three asparagine residues at positions 20, 92, and 324 of SEQ ID NO: 10 or positions 20, 92, and 337 of SEQ ID NO: 11.

According to further aspects of the invention, there is provided a polynucleotide encoding a modified Als3 protein of the invention.

According to further aspects of the invention, there is provided a vector comprising a polynucleotide encoding a modified Als3 protein of the invention.

5 According to further aspects of the invention, there is provided a host cell comprising: (1) one or more polynucleotide sequences that encode one or more heterologous glycosyltransferases; (2) a polynucleotide sequence that encodes a heterologous oligosaccharyl transferase; (3) a polynucleotide sequence that encodes a modified Als3 protein of the invention; and, optionally, (4) a polynucleotide sequence that encodes a polymerase.

10 According to further aspects of the invention, there is provided a method for producing a bioconjugate that comprises (or consists of) a modified Als3 protein linked to at least one saccharide antigen, the method comprising: (1) culturing a host cell of the invention under conditions suitable for the production of proteins; and (2) isolating the bioconjugate produced by said host cell, optionally isolating the bioconjugate from a periplasmic extract from the host cell.

15 According to further aspects of the invention, there is provided an immunogenic composition comprising a modified Als3 protein of the invention, a conjugate of the invention, or a bioconjugate of the invention and optionally a pharmaceutically acceptable excipient and/or carrier.

 According to further aspects of the invention, there is provided a method of making the immunogenic composition of the invention, the method comprising the step of mixing a modified Als3
20 protein of the invention, a conjugate of the invention, or a bioconjugate of the invention, with a pharmaceutically acceptable excipient or carrier.

 According to further aspects of the invention, there is provided a vaccine comprising an immunogenic composition of the invention and, optionally, a pharmaceutically acceptable excipient or carrier and optionally an adjuvant.

25 According to further aspects of the invention, there is provided a *Candida albicans* vaccine comprising: (1) a modified Als3 protein of the invention; (2) at least one *Candida albicans* saccharide antigen linked to said modified Als3 protein; and, optionally, (3) a pharmaceutically acceptable carrier or adjuvant.

 According to a further aspect of the invention, there is provided a method for treatment or
30 prevention of *Candida albicans* infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.

According to further aspects of the invention, there is provided a method for immunizing a subject against *Candida albicans* infection, the method comprising administering to the subject an immunoprotective dose of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of any of the invention, an immunogenic composition of the invention, or a vaccine of the invention.

According to further aspects of the invention, there is provided a method of inducing immune response to *Candida albicans* infection in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.

According to further aspects of the invention, there is provided a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention, for use in treatment or prevention of a disease caused by *Candida albicans* infection.

According to further aspects of the invention, there is provided a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention for use in the manufacture of a medicament for the treatment or prevention of a disease caused by *Candida albicans* infection.

According to further aspects of the invention, there is provided a method for increasing expression level of a modified Als3 protein of the invention, the method comprising substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein exhibits an increased expression level relative to a control Als3 protein which does not comprise one or more consensus sequences substituted for amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

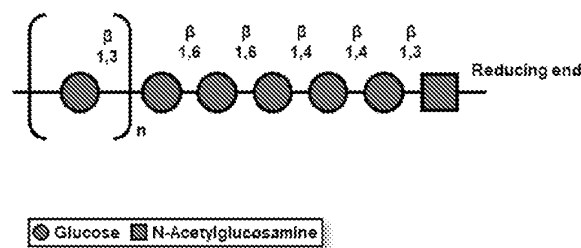
According to further aspects of the invention, there is provided a host cell comprising:

- i. a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;
- ii. A nucleotide sequence encoding a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule;
- iii. a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and

- iv. optionally, a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

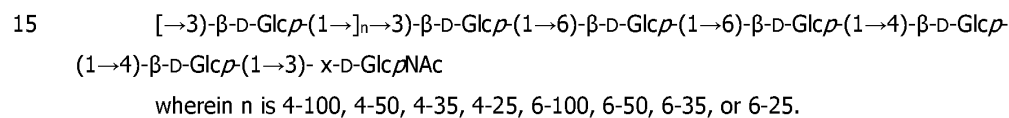
According to further aspects of the invention, there is provided a method of producing a glycoconjugate comprising a modified carrier protein and a β -1,3 glucan, wherein said method comprises culturing the host cell of the invention under conditions suitable for the production of proteins.

According to further aspects of the invention, there is provided a glucan having the structure:



wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

According to further aspects of the invention, there is provided a saccharide which is a glucan having the structure:



According to further aspects of the invention, there is provided a conjugate (*e.g.* bioconjugate) comprising a saccharide of the invention linked to an asparagine residue of a modified carrier protein.

According to further aspects of the invention, there is provided a host cell comprising a nucleotide sequence comprising (i) a wzm gene comprising a nucleotide sequence of SEQ ID NO: 36, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 36; and (ii) a wzt gene comprising a nucleotide sequence of SEQ ID NO: 37, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 37.

According to further aspects of the invention, there is provided a method of producing a β -1,3 glucan polymer in a prokaryotic host cell, the method comprising the steps of introducing and expressing in the host cell:

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- i. a nucleotide sequence encoding a first glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule, wherein the first glycosyltransferase is WfaP from *E. coli* O56;
 - ii. a nucleotide sequence encoding additional glycosyltransferases capable of synthesizing a fungal β -1, 3 glucan, wherein the additional glycosyltransferases comprise SleC, SleE, SleF, SleU and SleW from rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09, and wherein the host cell produces more SleW than SleC, SleE, SleF or SleU; and
 - iii. optionally, a nucleotide sequence encoding a translocase capable of translocating the β -1, 3 glucan to periplasmic side of an inner membrane of the prokaryotic host cell, wherein the translocase comprises Wzm-Wzt from *Klebsiella* sp., optionally from *Klebsiella pneumoniae*,

wherein the β -1,3 glucan polymer is linked to a lipid carrier via the GlcNAc and wherein the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules.

15 According to further aspects of the invention, there is provided a method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:

- a. obtaining a prokaryotic host cell of the invention that produces a β -1,3 glucan polymer; and
- b. further introducing and expressing in the host cell:
 - 20 i. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline, and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner
 - 25 membrane of the prokaryotic host cell; and
 - ii. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or
 - 30 *Campylobacter coli*.

DESCRIPTION OF DRAWINGS/FIGURES

FIG. 1 shows the structure of Als3-NT protein from *C. albicans* of SEQ ID NO: 27 (comprising residues 18-316 of SEQ ID NO: 1). Spheres indicate the position of insertion of glycosites.

5 **FIG. 2** shows the biosynthesis scheme for modified Als3-NT protein-glucan bioconjugate in *E. coli*.

FIG. 3 shows glycosylation tests with a series of modified Als3-NT proteins, each comprising a single glycosite.

FIG. 4 shows the expression levels and glycosylation levels of various modified Als3-NT proteins compared to the wild type Als3-NT protein. **FIG. 4A** shows the relative expression level of modified
10 Als3-NT proteins compared to the wild type Als3-NT protein. **FIG. 4B** shows the glycosylation efficiency of modified Als3-NT proteins.

FIG. 5 shows SDS-PAGE and Western Blot analyses of purified modified Als3-NT protein-glucan conjugates. **FIG. 5A**: SDS-PAGE analysis. **FIG. 5B**: Western Blot analysis with anti-Als3 antibody.

15 **FIG. 5C**: Western Blot analysis with anti-Fba antibody. **FIG. 5D**: Western Blot analysis with anti-Glucan antibody. **FIG. 5E**: Western Blot analysis with anti-Dectin antibody.

FIG. 6 shows Surface Plasma Resonance (SPR) assays for testing binding of wt Als3-NT protein ("Als³¹⁸⁻³¹⁶wt;" left panel), engineered unglycosylated modified Als3-NT protein ("uAls³¹⁸⁻³¹⁶-3S;" middle panel), and glycosylated modified Als3-NT protein ("β-glucan-Als³¹⁸⁻³¹⁶-3S;" right panel) to their natural ligand, fibronectin.

20 **FIG. 7** shows the preclinical testing of a modified Als3-NT protein-glucan bioconjugate (Als3-NT-3S-Fba_{bg}luc^{d+}; Als3-3FG) in rabbit. **FIG. 7A** shows the bioconjugate attributes. **FIG. 7B** shows a 3D representation of the modified Als3-NT protein-glucan bioconjugate. **Fig. 7C** shows a rabbit immunization scheme with a purified modified Als3-NT protein-glucan bioconjugate.

FIG. 8 shows the immunogenicity of the modified Als3-NT protein-glucan bioconjugate in rabbit. **FIG. 8A** shows that the modified Als3-NT protein-glucan bioconjugate is immunogenic. **FIG. 8B** shows that the Fba peptide (which is a part of the Als3-NT bioconjugate) is immunogenic.

FIG. 9 shows the immunogenicity of the modified Als3-NT protein-glucan bioconjugate in rabbit.

FIG. 10 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit adhesion of *C. albicans* hyphae to plastic.

30 **FIG. 11** shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit adhesion of *C. albicans* to vaginal epithelial cells. **FIG. 11A** shows the results of adhesion quantification. **FIG. 11B** shows a microscopy image of *Candida* adhered to epithelial cells.

FIG. 12 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to bind to *C. albicans* hyphae using whole cell ELISA.

35 **FIG. 13** shows a microscopy image of antibodies (against modified Als3-NT protein-glucan bioconjugate) bound to *C. albicans* cells.

FIG. 14 shows a microscopy image of antibodies (against modified Als3-NT protein-glucan bioconjugate) bound to *C. auris* VPCI479/P/13 cells.

FIG. 15 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit biofilm formation of *C. albicans* hyphae on 96-well plates.

- 5 **FIG. 16** shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to mediate neutrophil killing of *C. albicans* hyphae.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

- 10 As used herein, the term "Als3 protein or Als3" refers to a wild type Agglutinin-like sequence 3 protein comprising a wild type leader sequence (amino acid residues 1-17) at its N-terminus. In certain embodiments, the Als3 protein is from *Candida*, optionally from *Candida albicans*. In specific embodiments, the Als3 protein comprises the amino acid sequence of SEQ ID NO.: 1.

- As used herein, the term "Als3-NT protein or Als3-NT" refers to an N-terminal fragment of an Agglutinin-like sequence 3 (Als3) protein. In certain embodiments, the Als3-NT protein is from *Candida*, optionally from *Candida albicans*. In some embodiments, the Als3-NT protein comprises amino acid residues 18-316 of SEQ ID NO.: 1. In other embodiments, the Als3-NT protein comprises amino acid residues 18-329 of SEQ ID NO.: 1. In yet other embodiments, the Als3-NT protein comprises amino acid residues 1-316 of SEQ ID NO.: 1. In additional embodiments, the Als3-NT protein comprises amino acid residues 1-329 of SEQ ID NO.: 1. In other embodiments, the Als3-NT protein comprises an amino acid sequence selected from, but not limited to, the amino acid sequences of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, and SEQ ID NO: 41.
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- As used herein, the term "modified" protein refers to a protein that is altered (in one or more way) as compared to wild type protein (*e.g.* a "modified Als3 protein" excludes a wild type Als3 protein). In certain aspects, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acid residues of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In other embodiments, a modified Als3 protein refers to an Als3 protein that comprises a C-terminal or N-terminal deletion of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In yet other embodiments, a modified Als3 protein refers to an Als3 protein that comprises additions/deletions/substitutions of one or more amino acids of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In certain embodiments, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences (*e.g.* comprising
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an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline), wherein the one or more consensus sequences have been added next to or substituted for one or more amino acid residues of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1.

- 5 In other embodiments, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences (*e.g.* comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline), wherein the one or more consensus sequences have been added next to or substituted for one or more amino acid residues within amino acid residues 1-329 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%,
10 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 1-329 of SEQ ID NO: 1. In yet other embodiments, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences (*e.g.* comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline), wherein the one or more consensus sequences have been added next to or substituted for one or more amino acid residues within amino
15 acid residues 18-329 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-329 of SEQ ID NO: 1. In additional embodiments, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences (*e.g.* comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline), wherein the one or more
20 consensus sequences have been added next to or substituted for one or more amino acid residues within amino acid residues 1-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 1-316 of SEQ ID NO: 1. In specific embodiments, a modified Als3 protein refers to an Als3 protein that comprises one or more consensus sequences (*e.g.* comprising an amino acid sequence
25 of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline), wherein the one or more consensus sequences have been added next to or substituted for one or more amino acid residues within amino acid residues 18-316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%,
30 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1. In certain embodiments, a modified Als3 protein of the invention is an isolated modified Als3 protein. In other embodiments, a modified Als3 protein of the invention is a recombinant modified Als3 protein. In yet other embodiments, a modified Als3 protein of the invention is an isolated recombinant modified Als3 protein.

- As used herein, the term "modified Als3-NT protein or modified Als3-NT" refers to a Als3-NT
35 protein into which one or more glycosite sequences (*e.g.* D/E-X-N-Z-S/T) have been introduced. In certain embodiments, the modified Als3-NT protein is from *Candida*, optionally from *Candida albicans*.

In some embodiments, the modified Als3-NT protein comprises an amino acid sequence of SEQ ID NO: 10. In other embodiments, the modified Als3-NT protein comprises an amino acid sequence of SEQ ID NO: 11. In yet embodiments, the modified Als3-NT protein comprises an amino acid sequence selected from, but not limited to, the amino acid sequences of Mut1, Mut2, Mut3, Mut4, Mut5, Mut6, Mut7, Mut8, Mut9, Mut10, Mut11, Mut12, Mut13, Mut14, Mut15, Mut16, Mut17, and Mut18.

As used herein, the term "control Als3 protein" means, without limitation,: (1) Als3 protein that does not comprise one or more consensus sequences inserted next to or substituted for one or more amino acids of SEQ ID NO: 1 (or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1) or added to the N-terminal and/or C-terminal end of SEQ ID NO: 1 (or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1); (2) an Als3 protein that does not comprise one or more consensus sequences inserted next to or substituted for one or more amino acids of amino acid residues 18-316 of SEQ ID NO: 1 (or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1) or added to the N-terminal and/or C-terminal end of amino acid residues 18-316 of SEQ ID NO: 1 (or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1); (3) Als3 protein that does not comprise one or more consensus sequences inserted next to or substituted for one or more amino acids of amino acid residues 1-329 of SEQ ID NO: 1 (or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 1-329 of SEQ ID NO: 1) or added to the N-terminal and/or C-terminal end of amino acid residues 1-329 of SEQ ID NO: 1 (or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 1-329 of SEQ ID NO: 1); or (4) Als3 protein that does not comprise one or more consensus sequences inserted next to or substituted for one or more amino acids of amino acid residues 18-329 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-329 of SEQ ID NO: 1 or added to the N-terminal and/or C-terminal end of amino acid residues 18-329 of SEQ ID NO: 1 (or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-329 of SEQ ID NO: 1). Thus, a control Als3 protein includes, without limitation, a wild type Als3 protein, a wild type Als3 protein of SEQ ID NO: 1, an Als3 protein comprising amino acid residues 1-316 of SEQ ID NO: 1, an Als3 protein comprising amino acid residues 18-316 of SEQ ID NO: 1, an Als3 protein comprising amino acid residues 1-329 of SEQ ID NO: 1, an Als3 protein comprising amino acid residues 18-329 of SEQ ID NO: 1, and a modified Als3 protein of the invention that does not comprise one or more consensus sequences substituted for amino acids between amino acid residues 104-108 of amino acid residues

18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

As used herein, the term "carrier protein" refers to a protein which may be linked to an antigen (*e.g.* saccharide antigen, such as a fungal polysaccharide antigen) to create a conjugate (*e.g.* bioconjugate). A carrier protein activates T-cell mediated immunity in relation to the antigen to which it is conjugated. As used herein, the term "carrier protein" refers to a protein that comprises one or more consensus sequences to which a saccharide antigen of the invention is linked. In certain aspects, a carrier protein is a modified Als3 protein of the invention.

As used herein, the term "any amino acid except proline (pro, P)" refers to an amino acid selected from the group consisting of alanine (ala, A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

As used herein, the term "naturally occurring amino acid residues" refers to amino acids that are naturally incorporated into polypeptides. In particular, the 20 amino acids encoded by the universal genetic code: alanine (ala, A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

As used herein, the term "glycosyltransferases (GTFs, Gtfs)" refers to enzymes that establish glycosidic linkages. Glycosyltransferases are enzymes that catalyze the formation of the glycosidic linkage to form a glycoside. For example, they catalyze the transfer of saccharide moieties from an activated nucleotide sugar (also known as the "glycosyl donor") to a nucleophilic glycosyl acceptor molecule, the nucleophile of which can be oxygen-, carbon-, nitrogen-, or sulfur-based.

As used herein, the term "oligosaccharyl transferases (OTases or OSTs)" refers to enzymes that catalyze a mechanistically unique and selective transfer of an oligo- or polysaccharide (glycosylation) to the asparagine (N) residue at the consensus sequence of nascent or folded proteins. OSTs transfer of a 14-sugar oligosaccharide from dolichol to nascent protein. OST is a type of glycosyltransferase. The reaction catalyzed by OST is the central step in the N-linked glycosylation pathway. OST is a component of the translocon in the endoplasmic reticulum (ER) membrane. A lipid-linked core-oligosaccharide is assembled at the membrane of the endoplasmic reticulum and transferred to selected asparagine residues of nascent polypeptide chains by the oligosaccharyl transferase complex.

As used herein, the term "O-Antigens (also known as O-specific polysaccharides or O-side chains)" refers to a component of the surface lipopolysaccharide (LPS) of Gram-negative bacteria. Examples include O-antigens from *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

As used herein, the term "Lipopolysaccharide" or "LPS" refers to large molecules comprising a lipid and a polysaccharide joined by a covalent bond.

As used herein, the term "capsular polysaccharide (CP)" refers to a polysaccharide found on the bacterial cell wall. Examples include capsular polysaccharide from *Streptococcus pneumoniae*,
5 *Haemophilus influenzae*, *Neisseria meningitidis* and *Staphylococcus aureus*.

As used herein, the term "wzy" refers to a polysaccharide polymerase gene encoding an enzyme which catalyzes polysaccharide polymerization. The encoded enzyme transfers oligosaccharide units to the non-reducing end forming a glycosidic bond.

As used herein, the term "waaL" refers to a O antigen ligase gene encoding a membrane
10 bound enzyme. The encoded enzyme transfers undecaprenyl-diphosphate (UPP)-bound O antigen to the lipid A core oligosaccharide, forming lipopolysaccharide.

As used herein, the term "reducing end" refers to the reducing end of an oligosaccharide or polysaccharide is the monosaccharide with a free anomeric carbon that is not involved in a glycosidic bond and is thus capable of converting to the open-chain form.

As used herein, the term "conjugate" refers to carrier protein covalently linked to an antigen.
15

As used herein, the term "bioconjugate" refers to conjugate between a protein (*e.g.* a carrier protein) and an antigen (*e.g.* a saccharide antigen, such as a bacterial polysaccharide antigen) prepared in a host cell background, wherein host cell machinery links the antigen to the protein (*e.g.* N-linked glycosylation). Usually, in a bioconjugate the polysaccharide is linked to asparagine via N-acetylglucosamine.
20

As used herein, the term "immunogenic fragment" refers to a portion of an antigen smaller than the whole, that is capable of eliciting a humoral and/or cellular immune response in a host animal, *e.g.* human, specific for that fragment. Fragments of a protein can be produced using techniques
25 known in the art, *e.g.* recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Typically, fragments comprise at least 10, 20, 30, 40 or 50 contiguous amino acids of the full length sequence. Fragments may be readily modified by adding or removing 1, 2, 3, 4, 5,
30 6, 7, 8, 9, 10, 20, 30, 40 or 50 amino acids from either or both of the N and C termini. In certain aspects, a fragment of a modified Als3 protein of the invention still comprises the recited modifications that are made to the Als3 protein.

As used herein, the term "conservative amino acid substitution" involves substitution of a
35 native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge, hydrophobicity, or hydrophilicity of the amino acid residue at that position, and

without resulting in decreased immunogenicity. For example, these may be substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Conservative amino acid modifications to the sequence of a polypeptide (and the corresponding
5 modifications to the encoding nucleotides) may produce polypeptides having functional and chemical characteristics similar to those of a parental polypeptide.

As used herein, the term "deletion" refers to the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 1 to 6 residues (*e.g.* 1 to 4 residues) are deleted at any one site within the protein molecule.

10 As used herein, the terms "insertion" or "addition" (including other tenses thereof such as "inserted") refers to the addition of one or more non-native amino acid residues in the protein sequence or, as the context requires, addition of one or more non-native nucleotides in the polynucleotide sequence. Typically, no more than about from 1 to 10 residues, (*e.g.* 1 to 7 residues, 1 to 6 residues, or 1 to 4 residues) are inserted at any one site within the protein molecule.

15 As used herein, the term "added next to" refers to the addition of one or more non-native amino acid residues in the protein sequence at a position adjacent to the referenced amino acid or amino acid region. For example, "added next to one or more amino acids between amino acid residues 33-37" means the addition at a position adjacent to any one of amino acid residues 33-37 (including adjacent to amino acid residues 33 or 37).

20 As used herein, the term "glycosite" refers to an amino acid sequence recognized by a bacterial oligosaccharyl transferase, *e.g.* PglB of *Campylobacter jejuni*. Thus, a glycosite refers to an amino acid sequence within a carrier protein (*e.g.* a modified Als3 protein of the invention or a modified Als3-NT protein of the invention) to which an antigen saccharide (*e.g.* a β -1,3 glucan polymer) is linked, either covalently or non-covalently.

25 A "consensus sequence" refers to a sequence have a specific structure and/or function. As used herein, the term "consensus sequence" is a sequence comprising a glycosite. A consensus sequence of the invention includes, but is not limited to, a five amino acid consensus sequence D/E-X-N-Z-S/T, a seven amino acid consensus sequence K-D/E-X-N-Z-S/T-K, and an extended consensus sequence (*e.g.* J-U-B-D/E-X-N-Z-S/T-J-U-B).

30 As used herein, the term "introduced at" is used herein to reference the location and manner of inserting a consensus sequence into an amino acid sequence. A consensus sequence (or glycosite) which is introduced at an N-terminal or C-terminal position of a protein may be added next to the amino acid sequence at the N-terminus or C-terminus, whereas a consensus sequence (or glycosite) which is introduced at a specific amino acid residue within the protein (*e.g.* amino acid residue 18 of
35 SEQ ID NO: 1), may be substituted for that amino acid.

Unless specifically stated otherwise, providing a numeric range (*e.g.* "33-37") is inclusive of endpoints (*i.e.* includes the values 33 and 37). For example, "between amino acids 18 to 316 of SEQ ID NO: 1" refers to position in the amino acid sequence between amino acid 18 and amino acid 316 of SEQ ID NO: 1 including both amino acids 18 and 316.

5 The term "identical" or percent "identity" refers to nucleotide sequences or amino acid sequences that are the same or have a specified percentage of nucleotide residues or amino acid residues that are the same (*e.g.* 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identity over a specified region), when compared and aligned for maximum correspondence using, for example, sequence comparison algorithms or by manual alignment and visual inspection. Identity between
10 polypeptides may be calculated by various algorithms. In general, when calculating percentage identity the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. For example the Needleman Wunsch algorithm (Needleman and Wunsch 1970, J. Mol. Biol. 48: 443-453) for global alignment, or the Smith Waterman algorithm (Smith and Waterman 1981,
15 J. Mol. Biol. 147: 195- 197) for local alignment may be used, *e.g.* using the default parameters (Smith Waterman uses BLOSUM 62 scoring matrix with a Gap opening penalty of 10 and a Gap extension penalty of 1). A preferred algorithm is described by Dufresne *et al.* in Nature Biotechnology in 2002 (vol. 20, pp. 1269-71) and is used in the software GenePAST (Genome Quest Life Sciences, Inc. Boston, MA). The GenePAST "percent identity" algorithm finds the best fit between the query
20 sequence and the subject sequence, and expresses the alignment as an exact percentage. GenePAST makes no alignment scoring adjustments based on considerations of biological relevance between query and subject sequences. Identity between two sequences is calculated across the entire length of both sequences and is expressed as a percentage of the reference sequence (*e.g.* SEQ ID NO: 1 of the invention).

25 As used herein the term "recombinant" means artificial or synthetic. In certain embodiments, a "recombinant protein" refers to a protein that has been made using recombinant nucleotide sequences (nucleotide sequences introduced into a host cell). In certain embodiments, the nucleotide sequence that encodes a "recombinant protein" is heterologous to the host cell.

 As used herein the term "isolated" or "purified" refers to a protein, conjugate (*e.g.* bioconjugate), polynucleotide, or vector in a form not found in nature. This includes, for example, a
30 a protein, conjugate (*e.g.* bioconjugate), polynucleotide, or vector having been separated from host cell or organism (including crude extracts) or otherwise removed from its natural environment. In certain embodiments, an isolated or purified protein is a protein essentially free from all other polypeptides with which the protein is innately associated (or innately in contact with).

35 As used herein, the term "subject" refers to an animal, in particular a mammal such as a primate (*e.g.* human).

As used herein, the term "therapeutically or prophylactically effective amount," in the context of administering a therapy (*e.g.* an immunogenic composition or a vaccine of the invention) to a subject refers to the amount of a therapy which has a prophylactic and/or therapeutic effect(s). In certain embodiments, an "therapeutically or prophylactically effective amount" refers to the amount of a therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of a fungal infection or symptom associated therewith; (ii) reduce the duration of a fungal infection or symptom associated therewith; (iii) prevent the progression of a fungal infection or symptom associated therewith; (iv) cause regression of a fungal infection or symptom associated therewith; (v) prevent the development or onset of a fungal infection, or symptom associated therewith; (vi) prevent the recurrence of a fungal infection or symptom associated therewith; (vii) reduce organ failure associated with a fungal infection; (viii) reduce hospitalization of a subject having a fungal infection; (ix) reduce hospitalization length of a subject having a fungal infection; (x) increase the survival of a subject with a fungal infection; (xi) eliminate a fungal infection in a subject; (xii) inhibit or reduce a fungal replication in a subject; and/or (xiii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

As used herein, the term "immunoprotective dose," in the context of administering a therapy (*e.g.* an immunogenic composition or a vaccine of the invention) to a subject refers to the amount of a therapy which has a prophylactic and/or therapeutic effect(s). In certain embodiments, an "immunoprotective dose" refers to the amount of a therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of a fungal infection or symptom associated therewith; (ii) reduce the duration of a fungal infection or symptom associated therewith; (iii) prevent the progression of a fungal infection or symptom associated therewith; (iv) cause regression of a fungal infection or symptom associated therewith; (v) prevent the development or onset of a fungal infection, or symptom associated therewith; (vi) prevent the recurrence of a fungal infection or symptom associated therewith; (vii) reduce organ failure associated with a fungal infection; (viii) reduce hospitalization of a subject having a fungal infection; (ix) reduce hospitalization length of a subject having a fungal infection; (x) increase the survival of a subject with a fungal infection; (xi) eliminate a fungal infection in a subject; and/or (xii) inhibit or reduce a fungal replication in a subject.

The term "comprises" is open-ended and means "includes." Thus, unless the context requires otherwise, the word "comprises" or "has", and variations thereof (including "comprise" and "comprising" or "have" and "having", respectively), will be understood to imply the inclusion of a stated compound(s), molecule(s), composition(s), or steps, but not to the exclusion of any other compound(s), molecule(s), composition(s), or steps. The terms "comprising" and "having" when used as a transition phrase herein are open-ended whereas the term "consisting of" when used as a

transition phrase herein is closed (*i.e.*, limited to that which is listed and nothing more). In certain embodiments and for readability, the word "is" may be used as a substitute for "consists of" or "consisting of". The abbreviation, "*e.g.*" is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation "*e.g.*" is synonymous with the term "for example".

The term "conjugate vaccine" refers to a vaccine created by covalently linking a polysaccharide antigen to a carrier protein. Conjugate vaccine elicit immune response against a pathogen (*e.g.* a fungus) and immunological memory. In infants and elderly people, a protective immune response against polysaccharide antigens can be induced if these antigens are conjugated with proteins that induce a T-cell dependent response.

The term "glycoconjugate vaccine" refers to a vaccine comprising a protein carrier linked to an antigenic or immunogenic oligosaccharide.

As used herein, "undecaprenyl" or "und" refers to undecaprenol lipid composed of eleven prenol units. "Und-P" refers to undecaprenyl phosphate, which is a universal lipid carrier (derived from Und) of glycan biosynthetic intermediates for carbohydrate polymers. "Und-PP" refers to undecaprenyl pyrophosphate, which is a phosphorylated version of Und-P.

"Periplasmic space" or "periplasm" refers to the space between the inner cytoplasmic membrane and external outer membrane of a host cell (*e.g.* Gram-negative bacteria, *e.g.* *E. coli*).

The terms "of" and "from" are used herein interchangeably. Thus, a saccharide antigen "from" *Candida* means, without limitation, (1) a saccharide antigen obtained from *Candida* or (2) a saccharide antigen of *Candida*, *i.e.*, a saccharide antigen comprising a structure similar to a saccharide antigen from *Candida*, but, *e.g.*, produced recombinantly in a host cell (*e.g.* a bacterial cell).

sleC refers to a glycosyltransferase. In certain embodiments, sleC is a glycosyltransferase of *Agrobacterium* sp. ZX09. In some aspects, sleC is a wild type glycosyltransferase. In other aspects, sleC is a non-naturally occurring (*e.g.*, mutant and/or recombinant) glycosyltransferase.

sleE refers to a glycosyltransferase. In certain embodiments, sleE is a glycosyltransferase of *Agrobacterium* sp. ZX09. In some aspects, sleE is a wild type glycosyltransferase. In other aspects, sleE is a non-naturally occurring (*e.g.*, mutant and/or recombinant) glycosyltransferase.

sleF refers to a glycosyltransferase. In certain embodiments, sleF is a glycosyltransferase of *Agrobacterium* sp. ZX09. In some aspects, sleF is a wild type glycosyltransferase. In other aspects, sleF is a non-naturally occurring (*e.g.*, mutant and/or recombinant) glycosyltransferase.

sleU refers to a glycosyltransferase. In certain embodiments, sleU is a glycosyltransferase of *Agrobacterium* sp. ZX09. In some aspects, sleU is a wild type glycosyltransferase. In other aspects, sleU is a non-naturally occurring (*e.g.*, mutant and/or recombinant) glycosyltransferase.

sleW refers to a glycosyltransferase. In certain embodiments, sleW is a glycosyltransferase of *Agrobacterium* sp. ZX09. In some aspects, sleW is a wild type glycosyltransferase. In other aspects, sleW is a non-naturally occurring (e.g., mutant and/or recombinant) glycosyltransferase.

WfaP refers to a glycosyltransferase capable of covalently bonding a glucose to GlcNac. In certain embodiments, WfaP is a glycosyltransferase of *E. coli* 056. In some aspects, WfaP is a wild type glycosyltransferase. In other aspects, wfaP is a non-naturally occurring (e.g., mutant and/or recombinant) glycosyltransferase.

Wzm-Wzt refers to a translocase. In certain embodiments, Wzm-Wzt is a translocase of *Klebsiella pneumoniae*. In some aspects, Wzm-Wzt is a wild type translocase. In other aspects, Wzm-Wzt is a non-naturally occurring (e.g., mutant and/or recombinant) translocase.

PglB refers to a oligosaccharyl transferase. In certain embodiments, pglB is a oligosaccharyl transferase obtained from an organism including, but not limited to, *Campylobacter jejuni*, *Campylobacter coli*, or *Sinorhizobium meliloti* 1021. In certain embodiments, pglB is a oligosaccharyl transferase of *Campylobacter coli*. In some aspects, pglB is a wild type oligosaccharyl transferase. In other aspects, PglB is a non-naturally occurring oligosaccharyl transferase. In specific embodiments, the pglB of the invention comprises an amino acid sequence of SEQ ID NO: 20. In additional aspects, the pglB protein is an evolved pglB, i.e., an evolved oligosaccharyl transferase. By "evolved" is meant a protein or nucleic acid that has undergone directed evolution. Directed evolution is a method used is protein engineering that mimics the process of natural selection to steer proteins or nucleic acids toward a user-defined goal. The process of directed evolution consists of subjecting a gene to iterative rounds of mutagenesis (creating a library of variants), selection (expressing those variants and isolating members with the desired function) and amplification (generating a template for the next round). It can be performed in vivo (in living organisms), or in vitro (in cells or free in solution). Directed evolution is used both for protein engineering as an alternative to rationally designing modified proteins, as well as for experimental evolution studies of fundamental evolutionary principles in a controlled, laboratory environment. Thus, in certain embodiments, the pglB contains one or more mutations which enhances the activity of pglB for the saccharide antigen of the invention. In certain aspects, the pglB of the invention is an evolved pglB that transfers a saccharide antigen of the invention to a modified Als3 protein of the invention more efficiently in comparison to a wild-type pglB (e.g., a wild type pglB obtained from *Campylobacter jejuni*).

Als3 Protein

Agglutinin-like sequence 3 protein of *Candida albicans* (also known as "Als3") is a *C. albicans* hypha-specific cell surface protein that is a multifunctional adhesin and invasin, which enables *C. albicans* to adhere to biotic and abiotic surfaces, invade host cells, and obtain iron (Liu and Filler, 2011, *Eukaryotic Cell*, 10(2):168-173; Phan QT et al., 2007, *PLOS Biol.*, 5(3):e64). Als3 is a member of the agglutininlike sequence (Als) family of proteins and is encoded by the *ALS3* gene. At the N

terminus of the Als3 protein is a signal peptide ("SP") followed by a 300-amino-acid immunoglobulin-like domain ("NT") and a 104-amino-acid threonine-rich domain that contains β -sheets ("T") (Fig. 1A). The N-terminal domain of Als3 protein ("Als3-NT"), which contains a peptide binding cavity, is required for its adhesive function (Lin J et al., 2014, *J. Biol. Chem.*, 280(26):18401-18412). Antibodies obtained
 5 using Als3-NT domain for immunization block adherence of *Candida* to host endothelial and epithelial cells (Coleman, DA et al., 2009, *J. Mol. Meth.*). The central domain of the Als3 protein is composed of a variable number of 36-amino-acid tandem repeats ("TR") (Fig. 1A). These repeats are rich in serine and threonine, exposed on the cell surface, and required for adherence function. Because they
 10 are hydrophobic, the tandem repeats can directly mediate adherence to some substrates, such as polystyrene. The C terminus ("CT") of Als3 protein is serine and threonine rich and predicted to be heavily glycosylated. It contains a glycosylphosphatidylinositol anchorage sequence that is cleaved when the protein is covalently linked to the cell wall.

Functioning as an adhesin, Als3 mediates *C. albicans* invasion by attachment of the organism to epithelial cells, endothelial cells, and extracellular matrix proteins. It also plays an important role
 15 in biofilm formation on prosthetic surfaces, both alone and in mixed infection with *Streptococcus gordonii*. Als3 is one of two known *C. albicans* invasins. It binds to host cell receptors such as E-cadherin and N-cadherin and thereby induces host cells to endocytose the organism. Als3 also binds to host cell ferritin and enables *C. albicans* to utilize this protein as a source of iron.

Als3 is produced in *C. albicans* in a precursor from which a leader sequence of 17 amino acids
 20 ("signal peptide") is removed during the maturation process (Liu Y and Filler SG, 2011, *Eukaryot Cell*, 10(2):168-173). An Als3 protein useful in the invention can be produced by methods known in the art in view of the present disclosure, see for example (Gong J et al., 2019, *Antimicrob Agents Chemother*, 64(1):e01975-79).

In certain aspects of the invention, a full-length wild-type Als3 protein of *C. Albicans* comprises
 25 the amino acid sequence of SEQ ID NO: 1:

SEQ ID NO: 1: full-length wild-type Als3 protein sequence from *C. albicans* (with wild-type leader sequence underlined)

MLQOYTLLLIYLSVATAKTITGVFNSFNSLTWSNAATYNYKGPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCV
 30 FKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK
 CFTAGTNTVTFNDDGGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYSGTMGFANTYGDV
 QIDCSNIHVGITKGLNDWNPVSSSEFSYTKTSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCA
 GGYWQRAPFTLRWTGYRNSDAGSNGIVIVATTRTVTDSTTAVTTLPFDPNRDKTKTIEILKPIPTTTITTSYVGV
 TTSYLTKTAPIGETATVIVDIPYHTTTTTSKWTGTITSTTTHTNPTDSIDTVIVQVPLNPVTVTTEYWSQSFAT
 35 TTTITGPPGNTDVLIREPPNHTVTTTEYWSSEYTTTSTFTAPPGGTDVIIKEPPNPVTVTTEYWSSEYTTTTT
 TAPPGGTDVIIREPPNHTVTTTEYWSQSYTTTTTIVAPPGGTDVIIREPPNPVTVTTEYWSQSYATTTTTITAPP

GETDTVLIREPPNHTVTTTEYWSQSYATTTTITAPPGETDTVLIREPPNHTVTTTEYWSQSYTTTTVIAPPGGT
 DSVIIKEPPNPTVTTTEYWSQSYATTTTITAPPGETDTVLIREPPNHTVTTTEYWSQSYATTTTITAPPGETDTVL
 IREPPNHTVTTTEYWSQSFATTTTITAPPGGTDTVIIREPPNHTVTTTEYWSQSFATTTTIIAPPGETDTVLIREP
 PNPTVTTTEYWSQSYTTATTVTAPPGGTDTVIYDMSSEISSFSRPHYTNHTTLWSTTWVIETKITITETSEEG
 5 DKGCSWVSVSTRIVTIPNNIETPMVTNTVDTTTTTESTLQSPSGIFSESQSVETESSTFTTAQTNPSVPTTESEV
 FTTKGNNNGNGPYESPSTNVKSSMDENSEFTTSTAASSTDIENETIATTGSVEASSPIISSADETTTITTTAEST
 SVIEQQTNNNGGNAPSATSTSSPSTTTTANSDSVITSTTSTNQSQSQSNSDTQQTTLSQMTSSLVSLHMLT
 TFDGSGSVIQHSTWLCGLITLLSLFI

10 In certain embodiments, the present invention provides a modified Als3 protein. The term
 "modified Als3 protein" refers to a Als3 protein comprising an amino acid sequence (for example,
 having a amino acid sequence of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1), which Als3 amino
 acid sequence has been modified by the addition, substitution or deletion of one or more amino acids
 15 (for example, by addition of a consensus sequence(s) selected from D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T
 and/ or an extended consensus sequence (e.g. J-U-B-D/E-X-N-Z-S/T-J-U-B); and/or by substitution of
 one or more amino acids by a consensus sequence(s) selected from D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T.
 For example, a modified Als3 protein may be an Als3 amino acid sequence of SEQ ID NO: 1 which has
 been modified in that the amino acid sequence comprises one or more consensus sequences selected
 20 from from D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T and/or an extended consensus sequence (e.g. J-U-B-D/E-
 X-N-Z-S/T-J-U-B). As used herein, in consensus sequences of the present invention X and Z are
 independently any amino acid except proline; preferably, X is Q (glutamine) and Z is A (alanine). In
 certain embodiments, a modified Als3 protein of the invention may comprise further modifications
 (e.g., additions, substitutions, and/or deletions of one or more amino acid residues). In specific
 25 embodiments, a modified Als3 protein of the invention comprises a C-terminal deletion of the amino
 acid sequence of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%,
 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1. Thus, in certain
 aspects, a modified Als3 protein of the invention comprises amino acid residues 1-316 of SEQ ID NO.:
 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical
 30 to amino acid residues 18-316 of SEQ ID NO: 1. In other aspects, a modified Als3 protein of the
 invention comprises amino acid residues 1-329 of SEQ ID NO.: 1 or an amino acid sequence at least
 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of
 SEQ ID NO: 1. In additional aspects, a modified Als3 protein of the invention comprises amino acid
 residues 18-329 of SEQ ID NO.: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%,
 35 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1. In preferred
 embodiments, a modified Als3 protein of the invention comprises amino acid residues 18-316 of SEQ

ID NO.: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In some embodiments, the modified Als3 protein of the invention is a non-naturally occurring Als3 protein (*i.e.* not native). In certain embodiments, a modified Als3 protein of the invention may have an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1. In some aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 80% identical to SEQ ID NO: 1. In other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 85% identical to SEQ ID NO: 1. In yet other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 90% identical to SEQ ID NO: 1. In still other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 91% identical to SEQ ID NO: 1. In additional aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 92% identical to SEQ ID NO: 1. In other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 93% identical to SEQ ID NO: 1. In still other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 94% identical to SEQ ID NO: 1. In yet other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 95% identical to SEQ ID NO: 1. In certain aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 96% identical to SEQ ID NO: 1. In other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 97% identical to SEQ ID NO: 1. In additional aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 98% identical to SEQ ID NO: 1. In yet other aspects, a modified Als3 protein of the invention may have an amino acid sequence at least 99% identical to SEQ ID NO: 1.

In certain aspects, a modified Als3 protein of the invention comprises one or more consensus sequences. The terms "glycosite sequence", "consensus glycosite sequence" and "consensus sequence" are used herein interchangeably. In certain aspects, a modified Als3 protein of the invention comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten consensus sequences. In other aspects, a modified Als3 protein of the invention contains one, two, three, four, five, six, seven, eight, nine, or ten consensus sequences. In specific aspects, a modified Als3 protein of the invention contains at least three consensus sequences. In preferred aspects, a modified Als3 protein of the invention contains three consensus sequences. In certain embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein all of the consensus sequences have identical amino acid sequences. In other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein all of the consensus sequences have different amino acid sequences. In yet other embodiments, a modified Als3 protein of the invention comprises one or

more consensus sequences, wherein at least two of the consensus sequences have identical amino acid sequences.

Thus, in certain embodiments, the present invention provides a modified Als3 protein comprising amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence at least 80%,
5 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, modified in that the amino acid sequence comprises one or more consensus sequences comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

In certain aspects, a modified Als3 protein of the invention comprises one or more consensus
10 sequences, wherein the one or more consensus sequences have each been added next to or substituted for one or more amino acids, selected from specific amino acid residues within a modified Als3 protein of the invention (consensus sequence sites). These one or more consensus sequence sites are independently selected from (1) one or more amino acids between amino acid residues 18-23 (*e.g.* amino acid residue 18), (2) one or more amino acids between amino acid residues 311-316
15 (*e.g.* amino acid residue 316), (3) one or more amino acids between amino acid residues 28-42 (*e.g.* one or more amino acids between amino acid residues 33-37), (4) one or more amino acids between amino acid residues 75-87 (*e.g.* one or more amino acids between amino acid residues 80-82), (5) one or more amino acids between amino acid residues 82-92 (*e.g.* amino acid residue 87), (6) one or more amino acids between amino acid residues 99-113 (*e.g.* one or more amino acids between amino
20 acid residues 104-108), (7) one or more amino acids between amino acid residues 114-126 (*e.g.* one or more amino acids between amino acid residues 119-121), (8) one or more amino acids between amino acid residues 118-132 (*e.g.* one or more amino acids between amino acid residues 123-127), (9) one or more amino acids between amino acid residues 150-164 (*e.g.* one or more amino acids between amino acid residues 155-159), (10) one or more amino acids between amino acid residues
25 158-169 (*e.g.* one or more amino acids between amino acid residues 163-164), (11) one or more amino acids between amino acid residues 163-177 (*e.g.* one or more amino acids between amino acid residues 168-172), (12) one or more amino acids between amino acid residues 170-184 (*e.g.* one or more amino acids between amino acid residues 175-179), (13) one or more amino acids between amino acid residues 202-212 (*e.g.* amino acid residue 207), (14) one or more amino acids between
30 amino acid residues 215-225 (*e.g.* amino acid residue 220), (15) one or more amino acids between amino acid residues 231-242 (*e.g.* one or more amino acids between amino acid residues 236-237), (16) one or more amino acids between amino acid residues 265-275 (*e.g.* amino acid residue 270), (17) one or more amino acids between amino acid residues 271-281 (*e.g.* amino acid residue 276), (18) one or more amino acids between amino acid residues 281-292 (*e.g.* one or more amino acids
35 between amino acid residues 286-287), and (19) one or more amino acids between amino acid residues 294-305 (*e.g.* one or more amino acids between amino acid residues 299-300) of SEQ ID

NO: 1 or at equivalent position(s) within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1.

In certain aspects, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted
5 for one or more amino acids selected from the group consisting of: one or more amino acids between amino acid residues 18-23, one or more amino acids between amino acid residues 28-42, one or more amino acids between amino acid residues 75-87, one or more amino acids between amino acid residues 82-92, one or more amino acids between amino acid residues 99-113, one or more amino acids between amino acid residues 114-126, one or more amino acids between amino acid residues
10 118-132, one or more amino acids between amino acid residues 150-164, one or more amino acids between amino acid residues 158-169, one or more amino acids between amino acid residues 163-177, one or more amino acids between amino acid residues 170-184, one or more amino acids between amino acid residues 202-212, one or more amino acids between amino acid residues 215-225, one or more amino acids between amino acid residues 231-242, one or more amino acids
15 between amino acid residues 265-275, one or more amino acids between amino acid residues 271-281, one or more amino acids between amino acid residues 281-292, and one or more amino acids between amino acid residues 294-305 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

20 In certain embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 18 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

25 In other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 33-37 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

30 In some embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 80-82 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

35 In yet other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or

substituted for amino acid residue 87 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

5 In certain embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

10 In certain aspects, substitution of the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1 results in an increase in expression level of the modified Als3 protein relative to a control Als3 protein. In some aspects, the expression level of the modified Als3 protein is increased at least about 2-fold, at least about 3-fold, at least about 4-
15 fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold relative to the control Als3 protein. In other aspects, the expression level of the modified Als3 protein is increased about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, or about 10-fold relative to the control Als3 protein. In specific embodiments, substitution of the one or more consensus sequences
20 for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1 results in an increase in expression level of the modified Als3 protein of about 4-fold relative to a control Als3 protein.

In additional embodiments, a modified Als3 protein of the invention comprises one or more
25 consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 119-121 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In other aspects, a modified Als3 protein of the invention comprises one or more consensus
30 sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 123-127 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In yet other aspects, a modified Als3 protein of the invention comprises one or more consensus
35 sequences, wherein wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 155-159 of amino acid residues

18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In still other aspects, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 163-164 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In yet other aspects, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 168-172 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In additional aspects, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 175-179 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 207 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In yet other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 220 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In specific embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 236-237 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In certain embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 270 of amino acid residues 18-316 of SEQ ID NO: 1 or at an

equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In still other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 276 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In yet other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 286-287 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In additional embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 299-300 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In certain embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been substituted for (i) the amino acids between amino acid residues 33-37 and (ii) the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for (i) the amino acids between amino acid residues 33-37; (ii) the amino acids between amino acid residues 104-108; and (iii) amino acid residue 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In yet other embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for (i) the amino acids between amino acid residues 33-37; (ii) the amino acids between amino acid residues 104-108; and (iii) amino acid residue 329 of amino acid residues 18-329 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-329 of SEQ ID NO: 1.

In additional embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for (i) the amino acids between amino acid residues 33-37; (ii) the amino acids between amino acid residues 104-108; (iii) the amino acids between amino acid residues 163-164; (iv) amino acid residue 220; (v) the amino acids between amino acid residues 299-300; and (vi) amino acid residue 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In further embodiments, a modified Als3 protein of the invention comprises one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for (i) the amino acids between amino acid residues 33-37; (ii) the amino acids between amino acid residues 104-108; (iii) the amino acids between amino acid residues 163-164; (iv) amino acid residue 220; (v) the amino acids between amino acid residues 299-300; and (vi) amino acid residue 329 of amino acid residues 18-329 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-329 of SEQ ID NO: 1.

In certain embodiments, the modified Als3 protein of the invention is from a fungi. In certain aspects, the fungi is *Candida*. Thus, in some embodiments, the modified Als3 protein of the invention is from *Candida*. In certain aspects, the *Candida* includes, but is not limited to, *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanaea* and *Candida tropicalis*. In specific aspects, the modified Als3 protein of the invention is from *Candida albicans*. In certain embodiments, at least one of the one or more consensus sequences comprises an amino acid sequence of K-D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline. In certain aspects, X is Q (glutamine). In other aspects, Z is A (alanine). In some embodiments, the one or more consensus sequences include, but are not limited to, KDQNAT (SEQ ID NO: 5), KDQNAS (SEQ ID NO: 6) and DQNAT (SEQ ID NO: 7). In specific embodiments, X is Q (glutamine), Z is A (alanine) and the one or more consensus sequences are selected from the group consisting of KDQNAT (SEQ ID NO: 5), KDQNAS (SEQ ID NO: 6) and DQNAT (SEQ ID NO: 7).

In certain embodiments, a modified Als3 protein of the invention further comprises at least one Fructose biphosphate aldolase (Fba) peptide. The Fba peptide is a 14-mer peptide derived from the N-terminal portion of fructose-bisphosphate aldolase protein (Fba-1). Fba-1 protein is a multifunctional *C. albicans* cell wall protein and an important enzyme of glycolytic pathway. It can facilitate fungal attachment to human cells or abiotic surfaces, and protects *Candida* cells from the host's immune system (Elamin E, et al., 2021, *J. Immunol. Res.*, 2021:1-19). In addition to this, it promotes the detoxification of reactive oxygen species generated during respiratory burst. Proteomics analysis revealed that Fba1 is the most abundant and stable enzyme in *Candida* and is considered to

be one of the main immunodominant proteins (Elamin E, et al., 2021, *J. Immunol. Res.*, 2021:1-19). The Fba peptide has been previously been used to generate a self-adjuvanting vaccine (Xin H et al., 2012, *PLoS ONE*, 7:e35106). In certain aspects, the at least one Fba peptide comprises (or consists of) an amino acid sequence of YGKDVKDLFDYAE (SEQ ID NO: 3). In other aspects, the at least one
5 Fba peptide comprises (or consists of) an amino acid sequence at least 70%, 80%, 85%, 90%, or 92% identical to SEQ ID NO: 3.

In specific embodiments, a modified Als3 protein of the invention comprises at least one Fba peptide. In certain embodiments, the at least one Fba peptide comprises an amino acid sequence of YGKDVKDLFDYAE (SEQ ID NO: 3) or an amino acid sequence at least 70%, 80%, 85%, 90%, or
10 92% identical to SEQ ID NO: 3. In certain embodiments, the at least one Fba peptide is linked to a modified Als3 protein of the invention. In some embodiments, the at least one Fba peptide is non-covalently linked to a modified Als3 protein of the invention. In other embodiments, the at least one Fba peptide is covalently linked to a modified Als3 protein of the invention. In additional embodiments, the Fba peptide is linked to a modified Als3 protein of the invention at a single amino acid residue. In
15 other embodiments, the Fba peptide is linked to a modified Als3 protein of the invention at more than one amino acid residues. In additional embodiments, the Fba peptide is linked to a modified Als3 protein of the invention at one or more amino acid residues. In certain aspects, the one or more amino acid residues include, but are not limited to, amino acid residues 89, 163, 259, 199 and 316. In specific aspects, the Fba peptide is linked to a modified Als3 protein of the invention at amino acid
20 residue 316. The numbering of the amino acid residues as specified herein refers to the amino acid positions in SEQ ID NO: 1 (or where an amino acid sequence is at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 equivalent positions to that of SEQ ID NO: 1 if this sequence was lined up with an amino acid sequence of SEQ ID NO: 1 in order to maximise the sequence identity between the two sequences). In certain embodiments, the Fba peptide is covalently
25 linked to a modified Als3 protein of the invention at one or more amino acid residues selected from the group consisting of 89, 163, 259, 199 and 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent position(s) within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1. In specific embodiments, the Fba peptide is covalently linked to a modified Als3 protein of the invention at amino acid residue
30 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

In certain embodiments, a modified Als3 protein of the invention comprises at least one additional consensus sequence. In some embodiments, the at least one additional consensus
35 sequence has been added next to C-terminal amino acid residue of a modified Als3 protein of the invention. In other embodiments, a modified Als3 protein of the invention comprises at least one Fba

peptide and the at least one additional consensus sequence has been added next to C-terminal amino acid residue of the at least one Fba peptide. In additional embodiments, a modified Als3 protein of the invention comprises an Fba peptide comprising an amino acid sequence of YGKDVKDLFDYAQE (SEQ ID NO: 3) or an amino acid sequence at least 70%, 80%, 85%, 90%, or 92% identical to SEQ ID NO: 3, and the at least one consensus sequence has been added next to C-terminal amino acid residue of SEQ ID NO: 3 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 3. In specific embodiments, the modified Als3 protein comprises at least one additional consensus sequence comprising an amino acid sequence of J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise 1 to 5 naturally occurring amino acid residues, wherein the at least one additional consensus sequence has been added next to C-terminal amino acid residue of SEQ ID NO: 3 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 3. In certain embodiments, the at least one additional consensus sequence comprises an amino acid sequence of J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise one or more naturally occurring amino acid residues. In certain aspects, X is Q (glutamine). In other aspects, Z is A (alanine). In certain embodiments, J comprises at least one Glycine (G) residue. In some aspects, J comprises 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 Glycine (G) residues. In specific aspects, J comprises 1 to 5 Glycine (G) residues. In other aspects, B comprises at least one Glycine (G) residue. In certain aspects, B comprises 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 Glycine (G) residues. In specific aspects, B comprises 1 to 5 Glycine (G) residues. In additional embodiments, each of J and B comprises 1 to 5 Glycine (G) residues. In additional aspects, U comprises at least one Glycine (G) residue. In certain aspects, U comprises 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2 Glycine (G) residues. In specific aspects, U comprises 1 to 5 serine (S) residues. In some embodiments, X is Q, Z is A, each of J and B comprises 1 to 5 Glycine (G) residues and U comprises 1 to 5 serine (S) residues. In specific embodiments, the additional consensus sequence comprises (or consists of) an amino acid sequence of GSGGGDQ~~NAT~~GSGGG (SEQ ID NO: 9).

In certain embodiments, a modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 10:

SKTITGVFNSFNSLTW**KDQ**NATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQ
TSVDLTAHGVKYATCQFQAGEE**KDQ**NASTLTCTVSNTLTSPISIKALGTVTLPLAFNVGGTGSSVDLEDKCF
TNTVTFNDGGKKISINVD~~FERS~~NVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTMGFANTYGDVQIDCS
NIHVGITKGLNDWNPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTL~~SY~~ANEYTCAGGYW
QRAPFTLRWTGYRYGKDVKDLFDYAQE**GSGGGDQ**NAT**GSGGG**

In other embodiments, a modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 11:

SKTITGVFNSFNSLTWK**DQ**NATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQ
TSVDLTAHGKVKYATCQFQAGEE**KDQ**NA**ST**LTCTVSNTLTSPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAG
5 TNTVTFNDGGKKISINVDFFERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCS
NIHVGITKGLNDWNPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYW
QRAPFTLRWTGYRNSDAGSNGIVIVAYGKDVKDLFDYAE**GSGGGDQ**NAT**GSGGG**

In certain embodiments, a modified Als3 protein of the invention is glycosylated. In certain aspects, the modified Als3 protein of the invention is N-glycosylated.

10 The present inventors unexpectedly found that, when expressed in a host cell, a modified Als3 protein of the invention comprising at least one glycosite between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 ("Mut4 variant") showed a >2-fold increase in protein expression relative to control (*e.g.*, wild-type) Als3-NT expression. In additional embodiments, the present invention provides a method for increasing expression level of a modified Als3 protein of the
15 invention. In certain aspects, the method for increasing expression level of a modified Als3 protein of the invention comprises substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein
20 exhibits an increased expression level relative to a control Als3 protein.

In certain embodiments, the present invention provides a method for increasing expression level of a modified Als3 protein of the invention in a host cell, the method comprising substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at
25 least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein when expressed in a host cell exhibits an increased expression level relative to a control Als3 protein which does not comprise one or more consensus sequences substituted for amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%,
30 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

It will be understood by a person skilled in the art, that reference to "between amino acids ..." (for example "between amino acids 33-37") is referring to the amino acid number counting consecutively from the N-terminus of the amino acid sequence, for example "between amino acids 33 to 37...of SEQ ID NO: 1" refers to position in the amino acid sequence between amino acid 33 and
35 amino acid 37 of SEQ ID NO: 1 including both amino acids 33 and 37. Thus, in certain embodiments, where "one or more consensus sequences have been added next to or substituted for one or more

amino acids between amino acid residues 33-37 of SEQ ID NO: 1", the one or more consensus sequences may have been added next to or substituted for any one (or more) of amino acid numbers 33, 34, 35, 36, and 37 in SEQ ID NO: 1. A person skilled in the art will understand that when the Als3 amino acid sequence is a variant and/or fragment of an amino acid sequence of SEQ ID NO: 1, such as an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1, the reference to "between amino acids ..." refers to a position that would be equivalent to the defined position, if this sequence was lined up with an amino acid sequence of SEQ ID NO: 1 in order to maximise the sequence identity between the two sequences (Sequence alignment tools are not limited to Clustal Omega (www.ebi.ac.uk) MUSCLE (www.ebi.ac.uk), or T-coffee (www.tcoffee.org). In one aspect, the sequence alignment tool used is Clustal Omega (www.ebi.ac.uk).

The amino acid numbers referred to herein correspond to the amino acids in SEQ ID NO: 1 and as described above, a person skilled in the art can determine equivalent amino acid positions in an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 by alignment. The addition or deletion of amino acids from the variant and/or fragment of SEQ ID NO:1 could lead to a difference in the actual amino acid position of the consensus sequence in the mutated sequence, however, by lining the mutated sequence up with the reference sequence, the amino acid in an equivalent position to the corresponding amino acid in the reference sequence can be identified and hence the appropriate position for addition or substitution of the consensus sequence can be established.

In certain embodiments, a modified Als3 protein of the invention is an isolated modified Als3 protein. In other embodiments, a modified Als3 protein of the invention is a recombinant modified Als3 protein. In yet other embodiments, a modified Als3 protein of the invention is an isolated recombinant modified Als3 protein.

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Consensus sequence

In certain embodiments, a modified Als3 protein of the invention comprises a D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T or J-U-B-D/E-X-N-Z-S/T-J-U-B consensus sequence, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise 1 to 5 naturally occurring amino acid residues. The classical 5 amino acid glycosylation consensus sequence (D/E-X-N-Z-S/T) may be extended by 1-5 other amino acid residues either side of the consensus sequence for more efficient glycosylation J-U-B-D/E-X-N-Z-S/T-J-U-B (*e.g.* G-S-G-G-D/E-X-N-Z-S/T-G-S-G-G (SEQ ID NO: 2)). The classical 5 amino acid glycosylation consensus sequence (D/E-X-N-Z-S/T) may be extended by lysine residues for more efficient glycosylation (*e.g.* K-D/E-X-N-Z-S/T). Thus, consensus sequences in the modified Als3 protein of the invention may comprise (or consist of) a D/E-X-N-Z-S/T consensus sequence.

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In a modified Als3 protein of the invention, the consensus sequence(s) may be selected from: D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T (SEQ ID NO: 4) or J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X is Q (glutamine) and Z is A (alanine). In the modified Als3 protein of the invention, the consensus sequence(s) may be selected from: D/E-X-N-Z-S/T and K-D/E-X-N-Z-S/T (SEQ ID NO: 4), wherein X is Q (glutamine) and Z is A (alanine). In some embodiments, the consensus sequence is D/E-X-N-Z-S/T, wherein X is Q (glutamine) and Z is A (alanine), *e.g.* D-Q-N-A-T (SEQ ID NO: 7) also referred to as "DQNAT" (SEQ ID NO: 7). In other embodiments, the consensus sequence is K-D/E-X-N-Z-S/T (SEQ ID NO: 4), wherein X is Q (glutamine) and Z is A (alanine), *e.g.* K-D-Q-N-A-T (SEQ ID NO: 5) also referred to as "KDQNT" (SEQ ID NO: 5). In yet other embodiments, the consensus sequence is K-D/E-X-N-Z-S/T (SEQ ID NO: 4), wherein X is Q (glutamine) and Z is A (alanine), *e.g.* K-D-Q-N-A-S (SEQ ID NO: 6) also referred to as "KDQNAS" (SEQ ID NO: 6). In a modified Als3 protein of the invention, the consensus sequence(s) may be selected from: D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T (SEQ ID NO: 4) or J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X is Q (glutamine), Z is A (alanine), J, U and B are independently 1 to 5 amino acid residues independently selected from glycine and/or serine. In some embodiments, the consensus sequence is J-U-B-D/E-X-N-Z-S/T-J-U-B (SEQ ID NO: 8), wherein X is Q (glutamine), Z is A (alanine), each of J and B comprises 1 to 5 Glycine (G) residues and U comprises 1 to 5 serine (S) residues, *e.g.* G-S-G-G-G-D-Q-N-A-T-G-S-G-G-G (SEQ ID NO: 9) also referred to as "GSGGGDQNTGSGGG (SEQ ID NO: 9)".

In certain embodiments, the modified Als3 protein of the invention comprises at least two D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In other embodiment, the modified Als3 protein of the invention comprises at least three D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In yet other embodiments, the modified Als3 protein of the invention comprises at least four D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In still other embodiments, the modified Als3 protein of the invention comprises at least five D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In additional embodiments, the modified Als3 protein of the invention comprises at least six D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In further embodiments, the modified Als3 protein of the invention comprises at least seven D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In other embodiments, the modified Als3 protein of the invention contains three to seven D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In yet other embodiments, the modified Als3 protein of the invention contains four to seven D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences. In additional embodiments, the modified Als3 protein of the invention contains five to seven D/E-X-N-Z-S/T or K-D/E-X-N-Z-S/T consensus sequences.

Introduction of such glycosylation sites can be accomplished by, *e.g.* adding new amino acids to the primary structure of the protein (*i.e.* the glycosylation sites are added, in full or in part), or by mutating existing amino acids in the protein in order to generate the glycosylation sites (*i.e.* amino acids are not added to the protein, but selected amino acids of the protein are mutated so as to form

glycosylation sites). In certain embodiments, the consensus sequence(s) are recombinantly introduced into the Als3 amino acid sequence of SEQ ID NO: 1 or a Als3 amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1.

In some embodiments, a modified Als3 protein of the invention may further comprise a "peptide tag" or "tag", *i.e.* a sequence of amino acids that allows for the isolation and/or identification of the modified Als3 protein. For example, adding a tag to a modified Als3 protein of the invention can be useful in the purification of that protein and, hence, the purification of conjugate (*e.g.* bioconjugate) vaccines comprising the tagged modified Als3 protein. Exemplary tags that can be used herein include, without limitation, histidine (HIS) tags (*e.g.* hexa histidine-tag, or 6XHis-Tag), FLAG-TAG, and HA tags. In certain embodiments, the tag is a hexa-histidine tag. In certain aspects, the tags used herein are removable, *e.g.* removal by chemical agents or by enzymatic means, once they are no longer needed, *e.g.* after a modified Als3 protein of the invention has been purified. Thus, a modified Als3 protein of the invention may further comprise a peptide tag. In certain embodiments, the peptide tag is located at the C-terminus of the amino acid sequence of a modified Als3 protein of the invention. In some aspects, the peptide tag comprises six histidine residues at the C-terminus of the amino acid sequence of a modified Als3 protein of the invention. In certain embodiments, the present invention provides a modified Als3 protein comprising a tag (*e.g.*, a histidine tag). In other embodiments, the present invention provides a modified Als3 protein not comprising a tag (*e.g.*, a histidine tag), *e.g.*, a modified Als3 protein with the histidine tag removed. Thus, in particular aspects, the modified Als3 protein of the invention comprises (or consists of): (i) amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence which is at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or 100% identical to amino acid residues 18-316 of SEQ ID NO: 1 and (ii) a peptide tag (*e.g.* six histidine residues at the C-terminus of the amino acid sequence). In other aspects, a modified Als3 protein of the invention comprises (or consists of) amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence which is at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or 100% identical to amino acid residues 18-316 of SEQ ID NO: 1, with the peptide tag (*e.g.* histidine tag) removed.

In other embodiments, a modified Als3 protein of the invention comprises a signal sequence which is capable of directing the modified Als3 protein to the periplasm of a host cell (*e.g.* bacterium). Signal sequences, including periplasmic signal sequences, are usually removed during translocation of the protein into, for example, the periplasm by signal peptidases (*i.e.* a mature protein is a protein from which at least the signal sequence has been removed). The signal sequence is selected from, but is not limited to, *E. coli* flagellin (FlgI) [MIKFLSALILLVTTAAQA (SEQ ID NO: 21)], *E. coli* outer membrane porin A (OmpA) [MKKTAIAIAVALAGFATVAQA (SEQ ID NO: 22)], *E. coli* maltose binding protein (MalE) [MKIKTGARILALSALTMMFASALA (SEQ ID NO: 23)], *E. coli* outer membrane porin C (OmpC) [MKVKVLSLLVPALLVAGAANA] (SEQ ID NO: 24)], *Erwinia carotovora* pectate lyase (PelB)

[MKYLLPTAAAGLLLLAAQPAMA (SEQ ID NO: 63)], heat labile *E. coli* enterotoxin LTIIb [MSFKKIIKAFVIMAALVSVQAHA (SEQ ID NO: 64)], *Bacillus subtilis* endoxylanase XynA [MFKFKKKFLVGLTAAFMFSISMFSATASA (SEQ ID NO: 65)], *E. coli* DsbA [MKKIWLALAGLVAFSASA (SEQ ID NO: 66)], TolB [MKQALRVAFGFLILWASVLHA (SEQ ID NO: 67)] or SipA [MKMNKKVLLTSTMAASLLSVASVQAS (SEQ ID NO: 68)]. In specific embodiments, the signal sequence is from *E. coli* flagellin (FlgI) [MIKFLSALILLVTTAAQA (SEQ ID NO: 21)]. Thus, specific embodiments, the present invention provides a modified Als3 protein, wherein the amino acid sequence further comprises a signal sequence which is capable of directing the modified Als3 protein to the periplasm of a host cell (*e.g.* bacterium). In certain aspects, the signal sequence is FlgI. In some aspects, the FlgI comprises an amino acid sequence of SEQ ID NO: 21. In certain embodiments, the bacterial signal sequence is removed from the modified Als3 protein of the invention after the modified Als3 protein is transported to the periplasmic side of the inner membrane of a host cell of the invention.

In further embodiments, the present invention provides a polynucleotide encoding a modified Als3 protein of the invention. In certain embodiments, the present invention provides a polynucleotide encoding a modified Als3 protein of the invention, having a nucleotide sequence that encodes a polypeptide with an amino acid sequence that is at least 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 or 11. In some embodiments, the present invention provides a nucleotide sequence according to SEQ ID NO: 69, or a nucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 69. In additional embodiments, the present invention provides a nucleotide sequence according to SEQ ID NO: 70, or a nucleotide sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 70. In certain aspects, a nucleotide sequence of the invention comprises nucleotides encoding for amino acids corresponding to one (or more) consensus sequence(s) selected from: D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T-K, and J-U-B-D/E-X-N-Z-S/T-J-U-B. In other aspects, a nucleotide sequence of the invention comprises nucleotides encoding for amino acids corresponding to one (or more) consensus sequence(s) selected from: KDQNAT (SEQ ID NO: 5), KDQNAS (SEQ ID NO: 6), DQNAT (SEQ ID NO: 7), and GSGGGDQNATGSGGG (SEQ ID NO: 9). In particular aspects, a nucleotide sequence of the invention comprises nucleotides encoding for a modified Als3 protein of the invention comprising one or more consensus sequences, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids selected from the group consisting of: amino acid residue 18, one or more amino acids between amino acid residues 33-37, one or more amino acids between amino acid residues 80-82, amino acid residue 87, one or more amino acids between amino acid residues 104-108, one or more amino acids between amino acid residues 119-121, one or more amino acids between amino acid residues 123-127, one or more amino acids between amino acid residues 155-159, one or more amino acids between amino acid residues 163-164, one or more amino acids between amino acid residues 168-172, one or more amino acids between amino acid residues 175-

179, amino acid residue 207, amino acid residue 220, one or more amino acids between amino acid residues 236-237, amino acid residue 270, amino acid residue 276, one or more amino acids between amino acid residues 286-287, one or more amino acids between amino acid residues 299-300, and amino acid residue 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions
5 within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1. In specific aspects, a nucleotide sequence of the invention comprises nucleotides encoding for a modified Als3 protein of *Candida albicans* comprising one or more consensus sequences, wherein the one or more consensus sequences have been added next to one or more amino acids selected from the group consisting of: amino acid residue 16, amino
10 acid residue 88, and amino acid residue 321 of SEQ ID NO: 10 and amino acid residue 16, amino acid residue 88, and amino acid residue 334 of SEQ ID NO: 11.

In additional embodiments, the present invention provides a vector comprising a polynucleotide encoding a modified Als3 protein of the invention.

15 Conjugates

In certain embodiments, the present invention provides a conjugate comprising a modified Als3 protein of the invention. The conjugate of the invention may be a conjugate of a modified Als3 protein (*e.g.* chemical conjugate or bioconjugate). The conjugate of the invention may be a conjugate of a modified Als3 protein and an antigen, *e.g.* a saccharide antigen (*i.e.* bioconjugate). In specific
20 embodiments, the present invention provides a conjugate comprising (or consisting of) a modified Als3 protein of the invention and at least one saccharide antigen. In certain embodiments, the conjugate of the invention is a bioconjugate.

In some embodiments, the present invention provides a conjugate comprising a saccharide of the invention linked to a modified carrier protein. In certain aspects, the saccharide of the invention
25 is linked to an asparagine residue of the modified carrier protein. Thus, in specific embodiments, the present invention provides a conjugate comprising a saccharide of the invention linked to an asparagine residue of a modified carrier protein. In some embodiments, the conjugate is a bioconjugate. In certain aspects, the modified carrier protein of the invention includes, but is not limited to, Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B
30 of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural glycoprotein. In specific aspects, the modified carrier protein is a modified Als3 protein of the
35 invention.

In some embodiments, the modified Als3 protein of the invention is linked to the at least one saccharide antigen. In certain aspects, the modified Als3 protein of the invention is directly linked to the at least one saccharide antigen. In other aspects, the modified Als3 protein of the invention is linked to the at least one saccharide antigen through a linker. In certain embodiments, the modified

5 Als3 protein of the invention is non-covalently linked to the at least one saccharide antigen. In some aspects, the modified Als3 protein of the invention is non-covalently linked to the at least one saccharide antigen via an avidin-streptavidin interaction. In other embodiments, the modified Als3 protein of the invention is covalently linked to the at least one saccharide antigen through a chemical linkage obtainable using a chemical conjugation method (*i.e.* the conjugate is produced by chemical

10 conjugation). The chemical conjugation method may be selected from the group consisting of carbodiimide chemistry, reductive amination, cyanylation chemistry (for example CDAP chemistry), maleimide chemistry, hydrazide chemistry, ester chemistry, and N-hydroxysuccinimide chemistry. Conjugates can be prepared by direct reductive amination methods as described in, US200710184072 (Hausdorff) US 4365170 (Jennings) and US 4673574 (Anderson). Other methods are described in EP-

15 0-161-188, EP-208375 and EP-0-477508. The conjugation method may alternatively rely on activation of the saccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. Such conjugates are described in PCT published application WO 93/15760 Uniformed Services University and WO 95/08348 and WO 96/29094. See also Chu C. *et al.* Infect. Immunity, 1983 245 256.

20 In general the following types of chemical groups on a modified Als3 protein can be used for coupling / conjugation:

A) Carboxyl (for instance via aspartic acid or glutamic acid). In one embodiment this group is linked to amino groups on saccharides directly or to an amino group on a linker with carbodiimide chemistry *e.g.* with EDAC.

25 B) Amino group (for instance via lysine). In one embodiment this group is linked to carboxyl groups on saccharides directly or to a carboxyl group on a linker with carbodiimide chemistry *e.g.* with EDAC. In another embodiment this group is linked to hydroxyl groups activated with CDAP or CNBr on saccharides directly or to such groups on a linker; to saccharides or linkers having an aldehyde group; to saccharides or linkers having a succinimide ester group.

30 C) Sulphydryl (for instance via cysteine). In one embodiment this group is linked to a bromo or chloro acetylated saccharide or linker with maleimide chemistry. In one embodiment this group is activated/modified with bis diazobenzidine.

D) Hydroxyl group (for instance via tyrosine). In one embodiment this group is activated/modified with bis diazobenzidine.

35 E) Imidazolyl group (for instance via histidine). In one embodiment this group is activated/modified with bis diazobenzidine.

F) Guanidyl group (for instance via arginine).

G) Indolyl group (for instance via tryptophan).

On a saccharide, in general the following groups can be used for a coupling: OH, COOH or NH₂. Aldehyde groups can be generated after different treatments such as: periodate, acid hydrolysis,
5 hydroxyl peroxide, etc.

Conjugates can be purified by any method known in the art for purification of a protein, for example, by chromatography (*e.g.* ion exchange, anionic exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. See, *e.g.*, Saraswat *et al.*, 2013, Biomed. Res. Int. ID0312709 (p. 1-18); see
10 also the methods described in WO 2009/104074. The actual conditions used to purify a particular conjugate will depend, in past, on the synthesis strategy (*e.g.*, synthetic production vs. recombinant production) and on factors such as net charge, hydrophobicity, and/or hydrophilicity of the bioconjugate.

In some embodiments, the amino acid residue on the modified Als3 protein of the invention
15 to which the at least one saccharide antigen is linked includes, without limitation, Ala, Arg, Asp, Cys, Gly, Glu, Gln, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. In additional embodiments, the amino acid is: an amino acid comprising a terminal amine group, a lysine, an arginine, a glutaminic acid, an aspartic acid, a cysteine, a tyrosine, a histidine or a tryptophan. In certain embodiments, the amino acid residue on the modified Als3 protein of the invention to which the at least one saccharide
20 antigen is linked is not an asparagine residue and in this case, the conjugate is typically produced by chemical conjugation. In other embodiments, the at least one saccharide antigen is linked to an amino acid on a modified Als3 protein of the invention selected from asparagine, aspartic acid, glutamic acid, lysine, cysteine, tyrosine, histidine, arginine or tryptophan (*e.g.* asparagine) and in the case of asparagine, the conjugate may be a bioconjugate (for example an enzymatic conjugation using
25 a oligosaccharyl transferase such as PglB). In specific embodiments, the amino acid residue on a modified Als3 protein of the invention to which the at least one saccharide antigen is linked is an asparagine residue. In specific embodiments, the amino acid residue on the modified Als3 protein to which the at least one saccharide antigen is linked is part of the consensus sequence, *e.g.* the asparagine in D/E-X-N-Z-S/T, K-D/E-X-N-Z-S/T-K or J-U-B-D/E-X-N-Z-S/T-J-U-B consensus sequence.

30 In certain embodiments, the conjugate of the invention is a conjugate of a recombinant modified Als3 protein (*e.g.* chemical conjugate or bioconjugate). In other embodiments, the conjugate of the invention is a conjugate of an isolated recombinant modified Als3 protein and a recombinant antigen, *e.g.* recombinant saccharide antigen (*i.e.* bioconjugate).

In certain embodiments, the modified Als3 protein of the invention is linked to the at least
35 one saccharide antigen at one or more amino acid residues on the modified Als3 protein. In certain aspects, the one or more residues include, without limitations, one or more asparagine residues, one

or more aspartic acid residues, one or more glutamic acid residues, one or more lysine residues, one or more cysteine residues, one or more tyrosine residues, one or more histidine residues, one or more arginine residues, one or more tryptophan residues, one or more serine residues, and one or more threonine residues. In specific embodiments, the modified Als3 protein of the invention is linked to the at least one saccharide antigen at one or more asparagine residues on the modified Als3 protein of the invention. In certain aspects, the at least one saccharide antigen is linked to at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten asparagine residues of a modified Als3 protein of the invention. In some aspects, the at least one saccharide antigen is linked to one, two, three, four, five, six, seven, eight, nine, or ten asparagine residues of a modified Als3 protein of the invention. In certain embodiments, the at least one saccharide antigen is linked to at least three asparagine residues of a modified Als3 protein of the invention. In specific embodiments, the at least one saccharide antigen is linked to three asparagine residues of a modified Als3 protein of the invention. In certain aspects, the modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 10, and the three asparagine residues include, but are not limited to, positions 20, 92, and 324 of SEQ ID NO: 10. In other aspects, the modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 11, the three asparagine residues include, but are not limited to, positions 20, 92, and 337 of SEQ ID NO: 11. In additional embodiments, the at least one saccharide antigen is linked to at least one of three asparagine residues of a modified Als3 protein of the invention. In certain aspects, the modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 10, and the three asparagine residues include, but are not limited to, positions 20, 92, and 324 of SEQ ID NO: 10. In other aspects, the modified Als3 protein of the invention comprises (or consists of) an amino acid sequence of SEQ ID NO: 11, the three asparagine residues include, but are not limited to, positions 20, 92, and 337 of SEQ ID NO: 11. In certain embodiments, the present invention provides a modified Als3 protein of *Candida albicans* comprising (or consisting of): (1) an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11; and (2) at least one saccharide antigen of *Candida*, wherein the at least one saccharide antigen is linked to at least one of three asparagine residues at positions 20, 92, and 324 of SEQ ID NO: 10 or positions 20, 92, and 337 of SEQ ID NO: 11.

Antigens

The present invention provides conjugates (e.g., bioconjugates) wherein a carrier protein (e.g., a modified Als3 protein of the invention) may be linked (e.g., covalently or non-covalently linked) to a number of different antigens. In certain embodiments, the modified Als3 protein is linked to at least one antigen which is a saccharide antigen. In certain aspects, the antigen comprises at least one saccharide antigen. In some embodiments, the at least one saccharide antigen is a fungal

polysaccharide, a yeast polysaccharide or a mammalian polysaccharide. Polysaccharides comprise 2 or more monosaccharides, typically greater than 6, 8 or 10 monosaccharides.

In certain aspects, the at least one saccharide antigen in a conjugate (*e.g.* bioconjugate) of the invention includes, but is not limited to, O antigens of *E. coli*, *Salmonella* sp. O antigens, *Pseudomonas* sp., *Klebsiella* sp. O antigens, *Acinetobacter* O antigens, *Chlamydia trachomatis* O antigens, *Vibrio cholera* O antigens, *Listeria* sp. O antigens, *Legionella pneumophila* serotypes 1 to 15 O antigens, *Bordetella parapertussis* O antigens, *Burkholderia mallei* and *pseudomallei* O antigens, *Francisella tularensis* O antigens, *Campylobacter* sp. O antigens, capsular polysaccharides of *Clostridium difficile*, *Staphylococcus aureus* type 5 and 8, *Streptococcus pyrogenes*, *E. coli*, *Streptococcus agalacticae*, *Neisseria meningitidis*, *Candida* sp., *Candida albicans*, *Haemophilus influenza*, *Enterococcus faecalis* capsular polysaccharides type I-V, and other surface polysaccharide structures, *e.g.* the *Borrelia burgdorferi* glycolipids, *Neisseria meningitidis* pili O glycan and lipooligosaccharide (LOS), *Haemophilus influenza* LOS, *Leishmania major* lipophosphoglycan, tumor associated carbohydrate antigens, malaria glycosyl phosphatidylinositol, and *Mycobacterium tuberculosis* arabinomannan.

In certain embodiments, the at least one saccharide antigen is an O-antigen *e.g.* from a Gram-negative bacterium. In certain embodiments, the at least one saccharide antigen is an O-antigen from *Salmonella* species, *Shigella* species, *Pseudomonas* species or *Klebsiella* species. In other embodiments, the at least one saccharide antigen is an O-antigen from *Shigella* species, *Pseudomonas* species or *Klebsiella* species (*e.g.* *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Pseudomonas aeruginosa*, or *Klebsiella pneumoniae*). In other embodiments, the at least one saccharide antigen is an O-antigen from *Shigella dysenteriae*, *Shigella flexneri* or *Shigella sonnei*. For example, the antigen may be an O-antigen from *S. dysenteriae* type 1, *S. sonnei*, and *S. flexneri* type 6, and *S. flexneri* 2a and 3a O (Dmitriev, B.A., *et al.* Somatic Antigens of *Shigella* Eur J. Biochem, 1979. 98: p. 8; Liu et al Structure and genetics of *Shigella* O antigens FEMS Microbiology Review, 2008. 32: p. 27). In other embodiments, the at least one saccharide antigen is an O-antigen from *Pseudomonas aeruginosa*. For example, the antigen may be an O-antigen from *Pseudomonas aeruginosa* serotypes 1-20 (Raymond *et al.*, J Bacteriol. 2002 184(13):3614-22). In yet other embodiments, the at least one saccharide antigen is an O-antigen from *Klebsiella pneumoniae*.

In certain embodiments, the at least one saccharide antigen is a capsular polysaccharide from *Neisseria meningitidis* serogroup A (MenA), *N. meningitidis* serogroup C (MenC), *N. meningitidis* serogroup Y (MenY), *N. meningitidis* serogroup W (MenW), *H. influenzae* type b (Hib), Group B *Streptococcus* (GBS), *Streptococcus pneumoniae*, or *Staphylococcus aureus*. In other embodiments, the at least one saccharide antigen is a capsular polysaccharide from *Streptococcus* species or *Staphylococcus* species. (*e.g.* *Streptococcus pneumoniae* or *Staphylococcus aureus*). In additional embodiments, the at least one saccharide antigen is a capsular polysaccharide from *Staphylococcus*

aureus. For example, the at least one saccharide antigen may be a capsular polysaccharide from *Staphylococcus aureus* type 5 and 8. In other embodiments, the at least one saccharide antigen is a capsular polysaccharide from *Streptococcus pneumoniae*.

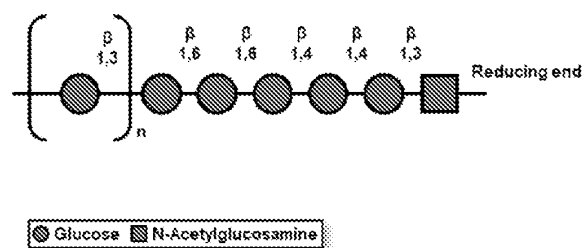
In additional embodiments, the at least one saccharide antigen is a fungal saccharide antigen.

- 5 In certain aspects, the fungi is *Candida* species. Thus, in certain aspects, the at least one saccharide antigen is a saccharide antigen of *Candida* species. In some aspects, the *Candida* species includes, but is not limited to, *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanaea* and *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*. In specific embodiments, the *Candida* species is *Candida albicans*. Thus, in specific embodiments, the at least
- 10 one saccharide antigen is a saccharide antigen of *Candida albicans*.

- In certain embodiments, the at least one saccharide antigen is a β -1,3 glucan polymer. Thus, in certain aspects, the at least one saccharide antigen is a β -1,3 glucan polymer of *Candida albicans*. β -1,3 glucans are widespread in nature. They are present in most yeasts and widespread in fungi and plants. *Candida* β -1,3 glucans consist of long linear polymers with occasional β -1,6 branchings.
- 15 They confer strength and shape to the cell wall. In certain embodiments, the β -1,3 glucan polymer of the invention is a naturally-occurring glucan. In other embodiments, the β -1,3 glucan polymer of the invention is a recombinantly produced glucan.

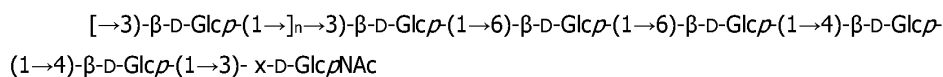
- In certain embodiments, the at least one saccharide antigen comprises a β -1,3 glucan polymer. In some aspects, the β -1,3 glucan polymer of the invention comprises at least four β -1,3
- 20 linked glucose molecules. In additional aspects, the β -1,3 glucan polymer comprises four to hundred, four to fifty, four to forty, four to thirty five, four to thirty, four to twenty five, four to twenty, four to ten, four to nine, four to eight, four to seven, four to six, four to five, four, five to hundred, five to fifty, five to forty, five to thirty five, five to thirty, five to twenty five, five to twenty, five to ten, five to nine, five to eight, five to seven, five to six, five, six to hundred, six to fifty, six to forty, six to thirty
- 25 five, six to thirty, six to twenty five, six to twenty, six to ten, six to nine, six to eight, six to seven, six, seven to hundred, seven to fifty, seven to forty, seven to thirty five, seven to thirty, seven to twenty five, seven to twenty, seven to ten, seven to nine, seven to eight, or seven β -1,3 linked glucose molecules. In some aspects, the β -1,3 glucan polymer comprises at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 11 consecutive β -1,3 linked glucose molecules.
- 30 In specific embodiments, the β -1,3 glucan polymer comprises at least 11 β -1,3 linked glucose molecules. In particular embodiments, the β -1,3 glucan polymer comprises at least 11 consecutive β -1,3 linked glucose molecules.

In some embodiments, the present invention provides a glucan having the structure:



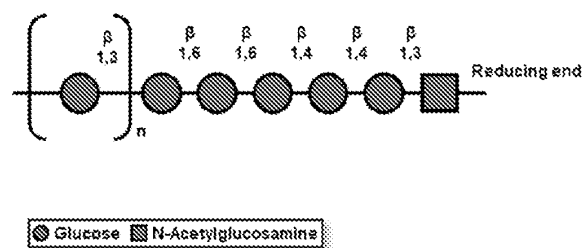
wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

- 5 In additional embodiments, the present invention provides a saccharide which is a glucan having the structure:



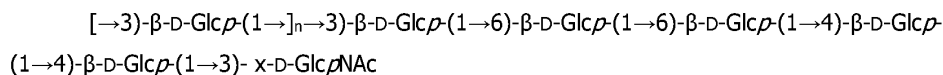
wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

- 10 In certain embodiments, the at least one saccharide antigen comprises a glucan having the structure:



- 15 wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

In other embodiments, the at least one saccharide antigen comprises a glucan having the structure:



- 20 wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

Thus, in certain embodiments, the present invention provides a modified Als3 protein of *Candida albicans* comprising (or consisting of): (1) an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11; and (2) at least one saccharide antigen of *Candida*, wherein the at least one saccharide antigen is a β -1,3 glucan polymer comprising (or consisting of) at least six consecutive β -1,3 linked glucose molecules, and wherein the at least one saccharide antigen is linked to at least one of three

asparagine residues at positions 20, 92, and 324 of SEQ ID NO: 10 or positions 20, 92, and 337 of SEQ ID NO: 11.

In other embodiments, the at least one saccharide antigen is a β -1,2 mannan polymer. In certain aspects, the at least one saccharide antigen is a β -1,2 mannan polymer of *Candida albicans*.
5 Mannans are the outermost layers of the cell wall and they participate in *Candida* cell adherence and immune system evasion. Mannans are highly complex and branched structures which are linked to secreted proteins. β -1,2 mannans are a part of *Candida*'s mannans present at the non-reducing end. Structures comprising at least two consecutive β -1,2 mannoses are exclusive to the *Candida* genus and are conserved in several *Candida* species (e.g., including *C. albicans*, *C. auris*, *C. guilliermondii*,
10 *C. lusitanae*, *C. tropicalis*, and excluding *C. glabrata*) (Morad HO et al, *Front Microbiol*, 2018). In certain embodiments, the β -1,2 mannan polymer of the invention is a naturally-occurring mannan. In other embodiments, the β -1,2 mannan polymer of the invention is a recombinantly produced mannan.

In certain embodiments, the at least one saccharide antigen comprises a β -1,2 mannan polymer. In some aspects, the β -1,2 mannan polymer comprises at least two β -1,2 linked mannose
15 molecules. In additional aspects, the β -1,2 mannan polymer comprises two to fifty, two to forty, two to thirty, two to twenty, two to ten, two to nine, two to eight, two to seven, two to six, two to five, two to four, two to three, two, three to fifty, three to forty, three to thirty, three to twenty, three to ten, three to nine, three to eight, three to seven, three to six, three to five, three to four, three, four to fifty, four to forty, four to thirty, four to twenty, four to ten, four to nine, four to eight, four to
20 seven, four to six, four to five, four, five to fifty, five to forty, five to thirty, five to twenty, five to ten, five to nine, five to eight, five to seven, five to six, or five, six to forty, six to thirty, six to twenty, six to ten, six to nine, six to eight, six to seven, six, seven to fifty, seven to forty, seven to thirty, seven to twenty, seven to ten, seven to nine, seven to eight, or seven 1,2 linked mannose molecules. In some aspects, the β -1,2 mannan polymer comprises at least 2, at least 3, at least 4, or at least 5
25 consecutive β -1,2 linked mannose molecules.

Host cell

In certain embodiments, the present invention provides host cells comprising a polynucleotide sequence that encodes a modified Als3 protein of the invention. In certain embodiments, the present
30 invention provides host cells that can be used to produce the bioconjugates of the invention. Host cells of the invention include, without limitation, archaea, prokaryotic host cells, and eukaryotic host cells. In certain embodiments, the host cell is a non-human host cell. In certain embodiments, the host cell is a eukaryotic host cell. In some aspects, the eukaryotic host cell includes, but is not limited to, a yeast cell, an insect cell and a mammalian cell. In additional embodiments, the host cell is a
35 prokaryotic host cell. In other aspects, the prokaryotic host cell is a bacterial cell. In some aspects, the bacteria is a gram positive bacteria. In other aspects, the bacteria is a gram negative bacteria.

In certain embodiments, the bacteria includes, but is not limited to, an *Escherichia* species, a *Shigella* species, a *Klebsiella* species, a *Xanthomonas* species, a *Salmonella* species, a *Yersinia* species, a *Lactococcus* species, a *Lactobacillus* species, a *Pseudomonas* species, a *Corynebacterium* species, a *Streptomyces* species, a *Streptococcus* species, a *Staphylococcus* species, a *Bacillus* species, and a *Clostridium* species. In some aspects, the bacteria is an *E. coli* species. In specific aspects, the bacteria is *E. coli*. Thus, exemplary host cells include, but are not limited to, an *Escherichia* species, a *Shigella* species, a *Klebsiella* species, a *Xanthomonas* species, a *Salmonella* species, a *Yersinia* species, a *Lactococcus* species, a *Lactobacillus* species, a *Pseudomonas* species, a *Corynebacterium* species, a *Streptomyces* species, a *Streptococcus* species, a *Staphylococcus* species, a *Bacillus* species, and a *Clostridium* species. In some aspects, the host cell is an *E. coli* species. In specific embodiments, the host cell is *E. coli*.

Host cells of the invention may be modified to delete or modify genes in the host cell genetic background (genome) that compete or interfere with the synthesis of the polysaccharide antigen of interest (*e.g.* compete or interfere with one or more heterologous polysaccharide synthesis genes that are recombinantly introduced into the host cell). These genes can be deleted or modified in the host cell background (genome) in a manner that makes them inactive/dysfunctional (*i.e.* the host cell nucleotide sequences that are deleted/modified do not encode a functional protein or do not encode a protein whatsoever). In certain embodiments, when nucleotide sequences are deleted from the genome of the host cells of the invention, they are replaced by a desirable sequence, *e.g.* a sequence that is useful for glycoprotein production. Exemplary genes that can be deleted in host cells (and, in some cases, replaced with other desired nucleotide sequences) include genes of host cells involved in glycolipid biosynthesis, such as *waaL* (see, *e.g.* Feldman et al. 2005, PNAS USA 102:3016-3021), the O antigen cluster (*rfb* or *wb*), enterobacterial common antigen cluster (*wec*), the lipid A core biosynthesis cluster (*waa*), galactose cluster (*gal*), arabinose cluster (*ara*), colonic acid cluster (*wc*), capsular polysaccharide cluster, undecaprenol-pyrophosphate biosynthesis genes (*e.g.* *uppS* (Undecaprenyl pyrophosphate synthase), *uppP* (Undecaprenyl diphosphatase)), Und-P recycling genes, metabolic enzymes involved in nucleotide activated sugar biosynthesis, enterobacterial common antigen cluster, and prophage O antigen modification clusters like the *gtrABS* cluster. In some embodiments, one or more of the *waaL* gene, *gtrA* gene, *gtrB* gene, *gtrS* gene, or a gene or genes from the *wec* cluster or a gene, or a gene or genes from the colonic acid cluster (*wc*), or a gene or genes from the *rfb* gene cluster are deleted or functionally inactivated from the genome of a prokaryotic host cell of the invention. In other embodiments, one or more of the *waaL* gene, *gtrA* gene, *gtrB* gene, *gtrS* gene, or a gene or genes from the *wec* cluster or a gene or genes from the *rfb* gene cluster are deleted or functionally inactivated from the genome of a prokaryotic host cell of the invention. In additional embodiments, the host cell of the invention is *E. coli*, wherein the native enterobacterial common antigen cluster (ECA, *wec*) with the exception of *wecA*, the colonic acid cluster

(*wca*), and the O16-antigen cluster have been deleted. In further embodiments, the native lipopolysaccharide O-antigen ligase *waaL* may be deleted from the host cell of the invention. In other embodiments, the native *gtrA* gene, *gtrB* gene and *gtrS* gene, may be deleted from the host cell of the invention.

5 The host cells of the present invention are engineered to comprise heterologous nucleotide sequences. In certain aspects, the host cells of the present invention are engineered to comprise a nucleotide sequence that encodes a modified Als3 protein of the invention, optionally within a plasmid. In other aspects, the host cells of the invention also comprise one or more nucleotide sequences comprising polysaccharide synthesis genes. Thus, host cells of the invention can produce a
10 bioconjugate comprising an antigen, for example a saccharide antigen (*e.g.* a fungal, bacterial, yeast or mammalian polysaccharide antigen) which is linked to a modified Als3 protein of the invention. In certain embodiments, one or more heterologous nucleotide sequences encode for the polysaccharide synthesis proteins to produce the fungal polysaccharide antigen, bacterial polysaccharide antigen, yeast polysaccharide antigen or mammalian polysaccharide antigen.

15 Thus, in certain embodiments, the present invention provides a host cell comprising: (1) one or more polynucleotide sequences that encode one or more heterologous glycosyltransferases; (2) a polynucleotide sequence that encodes a heterologous oligosaccharyl transferase; (3) a polynucleotide sequence that encodes a modified Als3 protein of the invention; and, optionally, (4) a polynucleotide sequence that encodes a polymerase.

20 In some aspects, the one or more heterologous glycosyltransferases includes, without limitation, exoL, exoM, exoO, exoU and exoW. In certain embodiments, the exoL, exoM, exoO, exoU and exoW are from a rhizobia. In certain aspects, the rhizobia is *Sinorhizobium*. In specific aspects, the *Sinorhizobium* is *Sinorhizobium meliloti* 1021. In certain embodiments, the one or more heterologous glycosyltransferases comprises exoL, exoM, exoO, exoU and exoW from a rhizobia,
25 optionally from *Sinorhizobium*, optionally from *Sinorhizobium meliloti* 1021. In other embodiments, the one or more heterologous glycosyltransferases includes, without limitation, SleC, SleE, SleF, SleU and SleW. In certain embodiments, the SleC, SleE, SleF, SleU and SleW are from a rhizobia. In certain aspects, the rhizobia is *Agrobacterium*. In specific aspects, the *Agrobacterium* is *Agrobacterium* sp. ZX09. In certain embodiments, the one or more heterologous glycosyltransferases
30 comprises SleC, SleE, SleF, SleU and SleW from a rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09.

In additional embodiments, the present invention provides a host cell comprising:

- i. a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;
- 35 ii. A nucleotide sequence encoding a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule;

- iii. a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and
- iv. optionally, a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

5 In yet other embodiments, the present invention provides a host cell comprising: (1) one or more polynucleotide sequences that encode one or more heterologous glycosyltransferases; (2) a polynucleotide sequence that encodes a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNac) molecule; (3) a polynucleotide sequence that encodes a heterologous oligosaccharyl transferase; (4) a polynucleotide sequence that encodes a modified Als3
10 protein of the invention; and, optionally, a polynucleotide sequence that encodes a polymerase.

In further embodiments, the present invention provides a host cell comprising:

- i. a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;
- ii. A nucleotide sequence encoding a glycosyltransferase capable of covalently
15 bonding a glucose molecule to an N-acetyl glucosamine (GlcNac) molecule;
- iii. a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and
- iv. optionally, a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline, optionally a
20 polynucleotide sequence of the invention.

In certain aspects, the modified carrier protein of the invention includes, but is not limited to, Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile
25 enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural glycoprotein. In specific aspects, the modified carrier protein is a modified Als3 protein of the invention.

In some embodiments, the one or more heterologous glycosyltransferases capable of
30 synthesizing a β -1,3 glucan polymer includes, without limitation, exoL, exoM, exoO, exoU and exoW. In certain embodiments, the exoL, exoM, exoO, exoU and exoW are from a rhizobia. In certain aspects, the rhizobia is *Sinorhizobium*. In specific aspects, the *Sinorhizobium* is *Sinorhizobium meliloti* 1021. In certain embodiments, the one or more heterologous glycosyltransferases comprises exoL, exoM, exoO, exoU and exoW from a rhizobia, optionally from *Sinorhizobium*, optionally from
35 *Sinorhizobium meliloti* 1021. In other embodiments, the one or more heterologous

glycosyltransferases includes, without limitation, SleC, SleE, SleF, SleU and SleW. In certain embodiments, the SleC, SleE, SleF, SleU and SleW are from a rhizobia. In certain aspects, the rhizobia is *Agrobacterium*. In specific aspects, the *Agrobacterium* is *Agrobacterium* sp. ZX09. In certain embodiments, the one or more heterologous glycosyltransferases of i. comprises SleC, SleE, SleF, SleU and SleW from a rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09.

In certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of exoL of *Sinorhizobium meliloti* 1021. In other aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleC of *Agrobacterium* sp. ZX09. In some aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence identical to the amino acid sequence of SleC of *Agrobacterium* sp. ZX09. In some aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence identical to the amino acid sequence of exoL of *Sinorhizobium meliloti* 1021. In certain embodiments, SleC of *Agrobacterium* sp. ZX09 comprises the amino acid sequence of SEQ ID NO: 12. Thus, in certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 12. In other aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence identical to SEQ ID NO: 12. In specific aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleC of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 12. In additional aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence identical to the amino acid sequence of SleC of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 12:

MIHILYLAHDLSDPAIRRRVLTLLAGGARVTLAGFRRGQNRLEIEGVVPVVLGETADGQFLQRMAAVA
KASLSLGKVLNGIPAPDVVLARNLEMLALAKRAMSIYSGRPALVYECLDIHRLLLHEGKPGQMLNAAQRYFARDA
KLLVTSSPAFVEHYFKPVSGLDLPVLLQENKVLALDDTTAATPRPRAPAPGEPWKIGWFGALRCRKSLEILAEFAR
RMEGRVEIILRGRPAYSEFADFDGFVAAAPHVHFHGPYKNPEDLAAIYNEVQFTWAIDFFEEGQNSSWLLPNRLY
EGCLYGTLPIALAGTETARFIEKRNIGFVLQQAGADDLAALFNRMTPTQTYADAFHTLSATDRKQWLTDRCRL
LVQQLSSLAKSASGHAREAQFSPV

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleC, optionally sleC from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence

encoding sleC from *Agrobacterium* sp. ZX09 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleC, optionally sleC from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence
 5 encoding sleC from *Agrobacterium* sp. ZX09 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34:

ATGATCCATATTCTCTACCTCGCGCATGATCTGTCAGACCCCGCCATTCGTCGGCGGGTGCTGACGC
 TGCTTGACAGGCGGGGCGCGGGTCACGCTGGCCGGTTTCCGGCGCGGACAGAACCGCTGGCGGAGATCGAA
 GCGCTCGTACCTGTCGTGCTCGGGGAAACCGCCGACGGGCAATTTCTGCAGCGCATGGCGGCAGTGGCGAA
 10 AGCCAGCCTTTCTGCTCGGCAAAGTCTTGAACGGAATTCGGCACCCGACGTCGTTCTGCCAGGAACCTCGA
 AATGCTGGCTCTGGCAAAGCGCGCCATGTCGATCTATTCCGGCCGCCGGCGCTGGTTTACGAATGTCTCGA
 TATTCATCGCCTGCTGCTCCACGAAGGCAAGCCCGACAGATGCTGAATGCCGCGCAGCGTTATTTGCGACG
 CGACGCAAAGCTTCTGGTGACAAGTTCCCGGCATTCTGGAGCATTATTTCAAGCCCGTGTGCGGCCTCGA
 CCTTCCTGTCCTGTTGCAGGAAAACAAGGTGCTGGCGCTCGACGACACCATTGCCGCCACGCCAAGACCACG
 15 GGCGCCCGCGCCCGGCGAACCATGGAATAATCGGCTGGTTCGGCGCGCTTCGCTGCCGCAAATCGCTTGAAA
 TTCTCGCTGAGTTGCTCGCCGATGGAAGGAGGGTCGAAATCATCCTGCGTGGCCGGCCGGCCTATTG
 GAGTTTGCTGATTTGATGGCTTCGTGGCGGCTGCTCCACATGTGCATTTCCACGGACCTTACAAAAACCC
 GAGGACCTAGCCGCATCTATAACGAGGTGCAATTTACCTGGGCGATCGACTTTTTCGAGGAAGGCCAGAAT
 TCCAGCTGGCTGCTGCCAACCAGCTTTACGAGGGCTGCCTTTACGGCACTCTGCCGATTGCACTTGCTGGC
 20 ACGGAAACGGCTCGCTTCATTGAAAAACGCAATATCGGCTTCGTCTGCAACAGGCGGGCGCTGACGATCTT
 GCCGCCCTCTTCAACCGGATGACCCCGCAAACCTATGCGGATGCCTTCCACACCCTGTCGGCAACAGACAGG
 AAACAATGGCTGACCGACCGCGACGATTGCCGTCTGCTGGTGCAGCAATTGTCCTCTCTCGCCAAATCCGCT
 TCCGGCCACGCCCGTGAAGCGCAGTTTTACCCGTGTAG

In certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing
 25 a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of exoM of *Sinorhizobium meliloti* 1021. In other embodiments, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleE of *Agrobacterium* sp. ZX09. In some aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to
 30 the amino acid sequence of SleE of *Agrobacterium* sp. ZX09. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of exoM of *Sinorhizobium meliloti* 1021. In certain embodiments, SleE of *Agrobacterium* sp. ZX09 comprises the amino acid sequence of SEQ ID NO: 13. Thus, in certain aspects, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or
 35 99% identical to SEQ ID NO: 13. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to SEQ ID NO: 13. In specific aspects, the one or more

heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleE of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 13. In additional aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of SleE of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 13:

MTDNTVTGTQYLKTVDIGICTYRRPALVATLLSLFELDVPEGVKVRLIVADNDEEPSAKASVDRLRETAP
FEITYVHCPKSNISIARNACLSECKADYLAFIDDDDETAPPHWLAALLEKADETGAETVLGPVTAVYRDNAPGWMK
RGDFHSTVPVWVNGEITGYTCNTLLRMEAPSVKGRRFALALGQSGGEDTHFFSHLHAAGGRIVFAEDAVLSEP
VPENRASFLWLAKRRFRSGQTHGRVLAEEKPGARRVVQVVKAGSKVLYCALFAALSGFNAVRRNRYALRGALHM
GSMGAFGVREIRQYGAVEAT

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleE, optionally sleE from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleE from *Agrobacterium* sp. ZX09 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 13, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleE, optionally sleE from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleE from *Agrobacterium* sp. ZX09 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 33:

ATGACCGACAACACCGTCACCGGCACGCAATATCTCAAGACCGTCGATATCGGCATCTGCACCTACA
GACGCCCGCGCTTGTGCCACACTTCTGTCACTCTTCGAGCTGGATGTGCCTGAAGGCGTGAAAGTTAGGC
TGATTGTCGCCGATAATGACGAGGAGCCAGCGCAAAGGCAAGCGTCGATCGCCTGCGCGAAACCGCCCCCT
TCGAGATCACCTATGTGCATTGCCGAAATCTAATATTTTCGATTGCCGCAATGCCTGCCTGTGCGAATGCAA
GGCGGACTATCTGCCTTTATCGATGACGACGAAACGGCGCCGCCCACTGGCTGGCCGCGCTTCTGGAAAA
GGCAGACGAGACCGGTGCAGAAACAGTTCTCGGCCCGTCACTGCGGTTTACCGGGAACGCGCCGGGCT
GGATGAAACGCGGAGATTTCACTCGACCGTTCCGGTCTGGGTCAATGGCGAGATCATCACCGTTATACAT
GCAACACGCTGCTCAGGATGGAAGCGCCATCGGTAAAGGGCCGGCGCTTCGCGCTGGCGCTTGGCCAGAGC
GGCGGCGAAGACACACATTTCTTCTCGCATCTCCACGCCGCCGGCGCCGTATCGTCTTTGCGGAAGACGCT
GTCTTGTGCGGAGCCGGTTCCGAAAAATCGGGCAAGCTTTCTATGGCTTGCCAAGCGCCGGTTCCGTTCCGGC
CAGACCCATGGACGGGTGCTGGCTGAAAAGAAACCCGGTGCAGCGCCGCGTCGTCCAGGTGGTGAAAGCTGG
TTTGAAAGTGCTTTATTGCGCTCTTTTGCCGCTTGAGCGGTTTCAATGCCGTACGCCGAACCGTTATGC
GTTGCGAGGCGCGCTGCATATGGGTTGATGAGCGGCGCCTTCGGTGTGCGCGAAATCCGGCAATATGGTG
CCGTGGAGGCGACCTGA

In certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of exoO of *Sinorhizobium meliloti* 1021. In other embodiments, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%,

98%, or 99% identical to the amino acid sequence of SleF of *Agrobacterium* sp. ZX09. In some aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of SleF of *Agrobacterium* sp. ZX09. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of exoO of *Sinorhizobium meliloti* 1021. In certain embodiments, SleF of *Agrobacterium* sp. ZX09 comprises the amino acid sequence of SEQ ID NO: 14. Thus, in certain aspects, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 14. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to EQ ID NO: 13. In specific aspects, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleF of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 14. In additional aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of SleF of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 14:

MENLTSPDISFVIAAYNAADTIEAAVQSALDQQGVTLIVVDDRSADDTIPFVEIAAIDPRVRLLE
ENRPGGGARNAGIEAATGRWIAVLDSDDVIRPERSACMMCRAEANADIAVDNLDVVYTDGRPMETMFPEEFL
EERPVLTLEDFISSNILFRSTFNFGYMKPMFRDRFLNNEALRFREDIRIGEDYILLASALAAGGLCVIEPKPGYIYNI
REGSISRVLLEHHVEAMMRADDEEFLSHYTLLPAAMDAQARARSLRLAHNFLTLENIKRRSVLGALKTTIRDPA
VLGHLRMPIAVRLRRLRDAVFAPAANTGVKRQIS

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleF, optionally sleF from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleF from *Agrobacterium* sp. ZX09 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 14, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleF, optionally sleF from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleF from *Agrobacterium* sp. ZX09 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 32:

ATGGAACCTCACCTCTCCGCCAGACATCAGCTTCGTTATCGCCGCCTATAATGCCGCCGATACGA
TTGAGGCCGCGGTTCAAAGCGCGCTCGATCAGCAGGGCGTGACGCTGGAAGTAATCGTCGTCGACGACCGC
TCCGCAGACGATACCATTCCTTCGTTGAAGCGATCGCCGCAATCGATCCGCGCGTGCGGCTGCTTGCCTC
GAAGAAACCGCGGTCCAGGCGGCGCCGCAACGCCGGCATCGAGGCCGCGACGGGGCGCTGGATCGCCGT
GCTCGACTCCGACGATGTCATCCGCCGAGCGCTCCGCCTGCATGATGTGCCGGGCGGAAGCCGCCAACG
CTGACATCGCTGTCGATAATCTCGATGTCGTCTACACCGATGGCCGGCCGATGGAGACGATGTTTCCCGAAG
AGTTTCTGGAGGAACGGCTGTTCTCACGCTGGAGGACTTCATTTCTCCAACATCCTGTTCCGCTCCACCTT
TAATCTCGGTACATGAAACCGATGTTCCGGCGCGATTTCCTTAATAACGAAGCACTGCGCTTCCGTGAGGA
TATCCGCATCGGCGAAGACTATATTCTCTCGCCTCGGCGCTTGCCGCTGGCGGCTCTGCGTCATCGAACC

GAAGCCGGGTTACATCTACAATATCCGCGAAGGTTTCGATTTCCCGTGTCTCGAACTTCATCACGTCGAAGC
 GATGATGCGGGCGGACGAAGAATTCTGAGCCACTACACCTTGCTGCCGGCCGCCATGGACGCGCAGCAGG
 CGAGAGCCCGCAGCCTGCGGCTGGCGCATAATTTTTTGAATTTGGTGAAAACATCAAGCGCCGCTCGGTAC
 TCGGCGCCTTGAAGACCACAATCCGAGATCTGCCGTGCTGGGGCACCTGAGAATGCCGATTGCAGTGCGG
 5 CTGAGGAGGCTGCGGGACGCCGTGTTTGCGCCCGCGCGAATACAGGGGTGAAAAGGCAGATTTTCATAA

In certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of exoU of *Sinorhizobium meliloti* 1021. In other embodiments, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%,
 10 98%, or 99% identical to the amino acid sequence of SleU of *Agrobacterium* sp. ZX09. In some aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of SleU of *Agrobacterium* sp. ZX09. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of exoU of *Sinorhizobium meliloti* 1021. In certain embodiments, SleU of *Agrobacterium* sp. ZX09
 15 comprises the amino acid sequence of SEQ ID NO: 15. Thus, in certain aspects, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 15. In other aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to SEQ ID NO: 15. In specific aspects, the one or more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or
 20 99% identical to the amino acid sequence of SleU of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 15. In additional aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino acid sequence of SleU of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 15:

MSVSGQPICVIAAKNASDTIDIAIRSALAEPEVGEVVVIDDGSTDTTSDVAHAADDGTGRLRVVRFDV
 25 NRGPSAARNHAISSAPLISILDADDDFFHGRFAAMLADDDWDLVADNIAFIQQSVPGASSMQPARFEPQARFL
 SLTEFVEGNISRPGVERGETGFLKPVIRRAFLDKHALRYDEALRLGEDYELYVRLAAGARYKVIRHCGYGAIVRG
 NSLSGRHRTEDLRLLYEADRAILAGCRLSAEETAILREHEKHIRAKFELRHFLDTKKQKGVSGALSHALARLPALP
 AITRGIWSDKTARFRKAAPVRDVRVLLDGTGPS

In certain embodiments, host cells of the present invention comprise a nucleotide sequence
 30 encoding sleU, optionally sleU from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleU from *Agrobacterium* sp. ZX09 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 15, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleU, optionally sleU from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence
 35 encoding sleU from *Agrobacterium* sp. ZX09 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31:

ATGGTGAGCGGTTACAGCCCATTTCGCTCATCATCGCTGCAAAGAATGCATCTGATACCATCGATA
 TCGCCATTCTGTTCCGCCCTTTCGCGAGCCAGAGGTCGGTGAAGTCGTGGTGATCGACGATGGTTCCACCGATA
 CAACGAGCGACGTGGCGCATGCGGCGGATGACGGCACGGGCCCTGCGGGTGGTGCGATTTCGACGTCAAC
 CGCGGGCCATCGGCTGCGCGCAATCATGCCATCTCGATTTCTCCGCTCCCCTCATCAGATTCTCGATGCG
 5 GACGATTTTTTCTTCCACGGCCGTTTCGCGGCCATGCTGGCCGATGACGACTGGGACCTCGTGGCCGATAAT
 ATCGCCTTCATTAGCAATCCGTTCCAGGCGCGTCTCCATGCAGCCTGCCCGCTTCGAGCCCCAGGCGCGG
 TTTTTGTCGCTGACGGAATTCGTGGAAGGCAATATTTCCAGGCCTGGTGTGGAGCGCGGGAAACCGGCTT
 CCTGAAACCGGTGATACGCCGCGCTTTCTCGACAAACATGCGCTGCGTTATGACGAGGCTCTGCGACTTGG
 CGAGGATTACGAATTTACGTGCGCGCGCTGGCGGCCGCGCGCTACAAGGTCATCCGTCATTGCGGTT
 10 ACGGTGCGATCGTTTCGCGGCAACTCGCTGAGTGGCCGCCACCGAACCGAAGATCTCCGTTCTCTACGAAG
 CAGACAGGGCGATTCTCGCCGTTGCCGCTCTCGGCAGAGGAAACGGCGATCCTGCGCGAGCACGAGAAA
 CACATCCGCGCCAAATTCGAGCTTCGCCATTTTCTCGATACAAAAAACAGAAGGGTGTGAGCGGAGCGCTG
 AGCCACGCCCTTGCGCTCTGCCGCCCTGCCGCCATTACAGCGGTATCTGGAGCGACAAGACCGCCCGC
 TTCCGCAAAGCGGCGCCGGTGCGGGACGTGCGTTATCTGCTGGACGGCACGCCGTTTCTCTAA

15 In certain aspects, the one or more heterologous glycosyltransferases capable of synthesizing
 a β -1,3 glucan polymer has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical
 to the amino acid sequence of exoW of *Sinorhizobium meliloti* 1021. In other aspects, the one or
 more heterologous glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%,
 or 99% identical to the amino acid sequence of SleW of *Agrobacterium* sp. ZX09. In some aspects,
 20 the one or more heterologous glycosyltransferases has an amino acid sequence identical to the amino
 acid sequence of SleW of *Agrobacterium* sp. ZX09. In other aspects, the one or more heterologous
 glycosyltransferases has an amino acid sequence identical to the amino acid sequence of exoW of
Sinorhizobium meliloti 1021. In certain embodiments, SleW of *Agrobacterium* sp. ZX09 comprises the
 amino acid sequence of SEQ ID NO: 16. Thus, in certain aspects, the one or more heterologous
 25 glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to
 SEQ ID NO: 16. In other aspects, the one or more heterologous glycosyltransferases has an amino
 acid sequence identical to SEQ ID NO: 16. In specific aspects, the one or more heterologous
 glycosyltransferases has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to
 the amino acid sequence of SleW of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 16. In additional
 30 aspects, the one or more heterologous glycosyltransferases has an amino acid sequence identical to
 the amino acid sequence of SleW of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 16:

LINARGETMARFTVIPYYQKHGVLGRALASVFAQTYQDFDLVIVDDSPYPIDQELAELSSEQKDRI
 LVIKQANAGPGGARNTGLDNVPDGTDYVAFLDSDDIWTPDHLRNAAFALTTYGGECYWASMQASDEFYHFAI
 SELEKNEGAARLSEKPLVIELPDLASVMLRNWSFLHLSCMVIGRPLFEKIRFDPALRLAAEDVLFCDLSILASKRTLL
 35 CDDAGAMRGMGVNIFHSIDNTSPEFLRQQFNTWVALDTLEGRFSRRPADVASIASYKNTARKQALWSQAGNLK
 RRKAPEFGLLLKWAMRDPALLRAAFELGAGKIVRSR

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleW, optionally sleW from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleW from *Agrobacterium* sp. ZX09 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 16, optionally within a plasmid.

5 In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding sleW, optionally sleW from *Agrobacterium* sp. ZX09, optionally a nucleotide sequence encoding sleW from *Agrobacterium* sp. ZX09 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 30:

TTGATCAACGCAAGGGGCGAAACAATGGCAAGATTTACTGTCGTCATTCCCTACTATCAAAAGCAGC
10 ACGGTGTCCTGGGACGTGCACTCGCATCGGTTTTTTCGCGAGACTTACCAGGACTTCGATCTTGTATCGTCG
ATGACGAATCGCCATACCCGATCGATCAGGAACTTGCGGAACTTTCGAGGAACAGAAAGACCGGATTCTTG
TCATTAAGCAGGCCAATGCCGGCCGGGCGGCGCCGCAACACCGGTCTGGACAATGTGCTGACGGCACC
GACTACGTTGCTTTCTTGATTCCGACGATATCTGGACGCCGATCATCTCGGAATGCGGCCTTTGCGCTC
ACGACCTATGGCGGCGAGTGCTACTGGGCGTCCATGCAGGCAAGTGACGAATTTTATTATCATTTCGCCATT
15 TCCGAGCTGGAGAAGAATGAGGGTGGCGCACGGCTTTTCGAGAAAGCCGCTGGTGATCGAACTGCCGGATCT
CGCAAGCGTCATGCTGCGGAACCTGGAGCTTCTGTCATCTCTCTGTCATGGTGATCGGTGCTCCTCTTTTCGA
GAAGATCCGTTTCGATCCGGCGCTCAGACTGGCGGCGGAAGACGTGCTGTTTTCTGCGATTCCATCCTTGC
ATCGAAGCGGACACTGCTCTGTGACGACGCCGGCGCGATGCGCGGCATGGGCGTCAATATCTTCCACAGCAT
CGACAATACCTCGCCGAATTCTGCGCCAGCAGTTCAATACCTGGGTGGCGCTCGATACGCTGGAGGGACG
20 TTTTCTCGCCGGCCGGCCGATGTGGCGTCCATTGCTTCTATAAAAAACCCGCGCGCAACAGGCTCTCTG
GAGCCAGGCTGGCAACCTGAAACGGCGCAAGGCTCCCGAATTCGGTTTGCTCCTAAAATGGGCAATGCGCGA
TCCGGCACTGCTGCGCGCCGCTTTCGAACTCGGCGCCGGAATAATCGTCCGCTCCAGATGA

In certain aspects, a host cell of the invention produces more SleW than SleC, SleE, SleF or SleU. In certain aspects, the host cell comprises one gene copy of SleW. In other aspects, the host
25 cell comprises more than one gene copy of SleW. In certain embodiments, the host cell comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten gene copies of SleW. In specific embodiments, the host cell contains at least two gene copies of a SleW of *Agrobacterium* sp. ZX09. In additional embodiments, the host cell contains at least one copy of a SleW of *Agrobacterium* sp. ZX09 under the control of a strong
30 promoter that enables higher expression of SleW compared to expression of SleC, SleE, SleF or SleU.

In certain embodiments, the glycosyltransferase capable of covalently bonding a glucose to GlcNac is WfaP. In certain aspects the WfaP is from a bacteria. In some aspects, the bacteria is a gram positive bacteria. In other aspects, the bacteria is a gram negative bacteria. In certain
embodiments, the bacteria includes, but is not limited to, an *Escherichia* species, a *Shigella* species,
35 *Klebsiella* species, a *Xanthomonas* species, a *Salmonella* species, a *Yersinia* species, a *Lactococcus* species, a *Lactobacillus* species, a *Pseudomonas* species, a *Corynebacterium* species, a *Streptomyces*

species, a *Streptococcus* species, a *Staphylococcus* species, a *Bacillus* species, and a *Clostridium* species. In some aspects, the bacteria is an *Escherichia* species. In certain aspects, the *Escherichia* species is *E. coli* 056. In specific embodiments, the glycosyltransferase capable of covalently bonding a glucose to GlcNac is WfaP from *E. coli* 056. In certain embodiments, the glycosyltransferase capable of covalently bonding a glucose to GlcNac has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of WfaP from *E. coli* 056. In other aspects, the glycosyltransferase capable of covalently bonding a glucose to GlcNac has an amino acid sequence identical to the amino acid sequence WfaP from *E. coli* 056. In certain embodiments, the WfaP from *E. coli* 056 comprises the amino acid sequence of SEQ ID NO: 17. Thus, in certain aspects, the glycosyltransferase capable of covalently bonding a glucose to GlcNac comprises an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 17. In other aspects, the glycosyltransferase capable of covalently bonding a glucose to GlcNac comprises an amino acid sequence identical to SEQ ID NO: 17. In specific aspects, glycosyltransferase capable of covalently bonding a glucose to GlcNac comprises an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of WfaP from *E. coli* 056 comprising SEQ ID NO: 17. In additional aspects, the glycosyltransferase capable of covalently bonding a glucose to GlcNac comprises an amino acid sequence identical to the amino acid sequence of WfaP from *E. coli* 056 comprising SEQ ID NO: 17:

MELVSIIAAYNCKDTIYATVESALSQTYKNIEIIICDDSSDDTWDIINKIKDSRIICIKNNYCKGAAGARNCALKI
AKGRYIAFLDSDDYWVTTKISNQIHFMETEKVFFSYSNYYIEKDFVITGVFSSPPEINYGAMLKYCNIACTVILDR
TGVKNISFPYIDKEDYALWLNILSKGIKARNTNLVDITYRVHAGSVSANKFKELIRQSNVLKSGIKAHHRHICLFY
YAINGLIKHCFSYRDKRNA

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding WfaP, optionally WfaP from *E. coli* 056, optionally a nucleotide sequence encoding WfaP from *E. coli* 056 comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 17, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding WfaP, optionally WfaP from *E. coli* 056, optionally a nucleotide sequence encoding WfaP from *E. coli* 056 comprising a nucleotide sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 35:

ATGGAAGTGGTCTCTATCATCATTGCCGCCTACAACTGTAAGGACACGATCTATGCCACCGTCGAATCCGCCC
TGAGCCAAACCTACAAGAACATCGAAATTATCATTTGCGATGACAGCTCTACCGATGACACGTGGGATATTAT
TAACAAAATCAAGGACAGCCGTATCATTTGCATCAAGAACAATACTGTAAGGGTGC GGCGGGCGCGCGTAA
CTGTGCTCTGAAAATCGCGAAGGGCCGCTATATTGCCTTTCTGGATTGAGTACTGAGTGGGTGACCACGAA
AATCTCGAACCAGATTCAATTCATGGAACCGAAAGGTGTTTTCTGAGTACTCGAATACTACATCGAAAAG
GATTTCTGTGATTACCGGCGTTTTCTAGTTCCCGCCGGAATCAACTATGGTGCTATGCTGAAATACTGCAATA

TCGCGTGTAGCACCGTTATTCTGGACCGCACGGGTGTCAAGAACATCTCTTTCCGTACATTGATAAGGAAG
 ACTACGCCCTGTGGCTGAATATCCTGTCAAAAGGCATTAAGGCACGTAACACCAATCTGGTCGATACCTATTA
 CCGCGTCCATGCCGGTAGCGTGTCTGCAAACAAATTCAAGGAACTGATTCGTCAAAGTAATGTTCTGAAATC
 CATCGGCATTAAGGCTCATCCGCATCATTTGCCTGTTTTATTACGCGATCAACGGTCTGATTAAACACTGT
 5 TTCTCCTATCGTGATAAGCGCAATGCCTAA

Oligosaccharyl Transferase

N-linked protein glycosylation (the addition of carbohydrate molecules to an asparagine residue in the polypeptide chain of the target protein) is the most common type of post-translational
 10 modification occurring in the endoplasmic reticulum of eukaryotic organisms. The process is accomplished by the enzymatic oligosaccharyl transferase complex (OST) responsible for the transfer of a preassembled oligosaccharide from a lipid carrier (dolichol phosphate) to an asparagine residue of a nascent protein within the conserved sequence Asn-X-Ser/Thr (where X is any amino acid except proline) in the Endoplasmic Reticulum.

15 It has been shown that a bacterium, the food-borne pathogen *Campylobacter jejuni*, can also N-glycosylate its proteins (Wacker *et al.* Science. 2002; 298(5599):1790-3) due to the fact that it possesses its own glycosylation machinery. The machinery responsible of this reaction is encoded by a cluster called "pgl" (for protein glycosylation). In certain embodiments, the *C. jejuni* glycosylation machinery is transferred to *E. coli* to allow for the glycosylation of recombinant proteins expressed by
 20 the host *E. coli* cells. Previous studies have demonstrated how to generate *E. coli* strains that can perform N-glycosylation (see, *e.g.* Wacker *et al.* Science. 2002; 298 (5599):1790-3; Nita-Lazar *et al.* Glycobiology. 2005; 15(4):361-7; Feldman *et al.* Proc Natl Acad Sci U S A. 2005; 102(8):3016-21; Kowarik *et al.* EMBO J. 2006; 25(9):1957-66; Wacker *et al.* Proc Natl Acad Sci U S A. 2006; 103(18):7088-93; International Patent Application Publication Nos. WO2003/074687,
 25 WO2006/119987, WO 2009/104074, and WO/2011/06261, and WO2011/138361).

Thus, in certain aspects, the host cells of the present invention comprise a nucleotide sequence encoding a heterologous oligosaccharyl transferase, optionally within a plasmid. In some embodiments, the heterologous oligosaccharyl transferase is a PglB. In certain embodiments, the PglB is from *Campylobacter*. In certain aspects, the *Campylobacter* includes, but is not limited to,
 30 *Campylobacter jejuni* or *Campylobacter coli*. In some aspects, the *Campylobacter* is *Sinorhizobium meliloti* 1021. In certain embodiments, the PglB is optionally from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli* comprising an amino acid sequence of SEQ ID NO: 20. In certain embodiments, the PglB comprises an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of PglB of *Campylobacter coli*. In other aspects, the PglB
 35 comprises an amino acid sequence identical to the amino acid sequence of PglB of *Campylobacter coli*. In certain embodiments, PglB of *Campylobacter coli* comprises the amino acid sequence of SEQ

ID NO: 20. In certain aspects, the PglB has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 20. In other aspects, the PglB has an amino acid sequence identical to SEQ ID NO: 20. In specific aspects, the PglB has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of PglB of *Campylobacter coli* comprising
 5 SEQ ID NO: 20. In additional aspects, the PglB has an amino acid sequence identical to the amino acid sequence of PglB of *Campylobacter coli* comprising SEQ ID NO: 20:

MLKKEYLKNPYLVLFAMIILAYVFSVFCRFYWWWASEFNEYFFNNQLMIISNDGYAFAEGARDMIAGF
 HQPNDSLSSYSSLSALTYWLYKITPFSFESIILYMSTFLSSLVVIPTILLANEYKRPLMGFVAALLASIANSYNRTM
 SGYYDTDMLVIVLPMFILFFMVRMILKKDFFSLIALPLFIGIYLWWYPSSYTLNVALIGLFLIYTLIFHRKEKIFYIAVI
 10 LSSLTLSNIAWFYQSAIIVILFALFALEQKRLNFMIIIGILGSATLIFLILSGGVDPIYQLKFYIFRSDESANLTQGF
 YFNVNQTIQEVENVLDSEFMRRISGSEIVFLSLFGFVWLLRKHKSMIMALPILVLGFLALKGGLRFTIYSVPVMAL
 GFGFLLSEFKAIMVKKYSQLTSNVCIVFATILTAPVFIHIYNYKAPT VFSQNEASLLNQLKNIANREDYVVTWWD
 YGYPVRYSDVKTLVDGGKHLGKDNFFPSFALSKDEQAAANMARLSVEYTEKSFYAPQNDILKTDILQAMMKDY
 NQSNVDLFLASLSKPDFKIDTPKTRDIYLYMPARMSLIFSTVASFSFINLDTGVLDKPFTSTAYPLDVKNGEIYLS
 15 NGVVLSDDFRSFKIGDNVSVNSIVEINSIKQGEYKITPIDDKAQFYIFYLKDSAIPYAQFILMDKTMFNASVQM
 FFLGNYDKNLFDLVINSRDAKVFKLKI

In certain embodiments, host cells of the present invention comprise a nucleotide sequence encoding PglB, optionally PglB from *Campylobacter jejuni*, optionally a nucleotide sequence encoding PglB from *Campylobacter jejuni* or *Campylobacter coli* comprising an amino acid sequence at least
 20 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 20, optionally within a plasmid.

In other embodiments, host cells of the present invention comprise a nucleotide sequence encoding PglB, optionally PglB from *Campylobacter jejuni*, optionally a nucleotide sequence encoding PglB from *Campylobacter jejuni* or *Campylobacter coli* comprising a nucleotide sequence at least 80%,
 25 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 29, optionally within a plasmid:

ATGCTGAAGAAAGAATATCTGAAAAATCCGTATCTGGTGCTGTTTCGCAATGATTATCCTGGCGTATG
 TGTTTAGCGTGTTCTGTCGTTTCTACTGGGTGTGGTGGGCAAGTGAATTTAACGAATATTTCTTTAACCAACC
 AGCTGATGATCATCTCCAATGATGGCTATGCCTTCGAGAAGGTGCCCGTGACATGATTGCAGGCTTTTCATC
 30 AGCCGAACGATCTGAGTTATTACGGTAGCTCTCTGTCCGCCCTGACCTATTGGCTGTACAAAATCACGCCGT
 TTAGTTTCGAATCCATTATCCTGTACATGAGTACCTTCCTGAGTTCCTGGTGGTTATTCGACGATCCTGCT
 GGCAAATGAATATAAACGTCCGCTGATGGGCTTTGTGGCGGCCCTGCTGGCTAGTATTGCGAACTCCTATTA
 CAATCGCACCATGAGTGTTATTACGATACGGACATGCTGGTCATTGTGCTGCCGATGTTTCATCCTGTTTTT
 CATGGTGCGTATGATTCTGAAAAAGGATTTCTTTAGCCTGATCGCTCTGCCGCTGTTTATTGGCATCTATCTG
 35 TGGTGGTACCCGTCATCGTATACCCTGAACGTTGCGCTGATTGGTCTGTTTCTGATTTACACGCTGATCTTCC
 ATCGCAAGGAAAAGATCTTTTATATCGCGTTATCCTGAGCTCTCTGACCCTGAGCAACATTGCTTGGTTTTA

TCAGTCTGCGATTATCGTCATCCTGTTTGCCCTGTTGCACTGGAACAAAAACGTCTGAATTTTCATGATTATC
GGCATTCTGGGTAGTGCTACCCTGATCTTTCTGATTCTGTCCGGCGGTGTTGATCCGATTCTGTACCAGCTG
AAATTTTATATCTTCCGCTCAGATGAATCGGCGAACCTGACCCAAGGCTTCATGTACTTCAACGTTAACCAGA
CGATCCAAGAAGTGGAAAATGTTGATCTGAGCGAATTTATGCGTCGCATTAGTGGCTCCGAAATCGTTTTTC
5 TGTCTCACTGTTTGGTTTCGTCTGGCTGCTGCGTAAACACAAGTCGATGATTATGGCCCTGCCGATCCTGG
TGCTGGGTTTCTGGCACTGAAAGGCGGTCTGCGCTTTACCATTTACAGCGTCCGGTCATGGCCCTGGGCT
TTGGTTTCTGCTGTCTGAATTTAAGGCAATCATGGTTAAAAAGTACTCACAGCTGACCTCGAACGCTGTCAT
TGTGTTCCGCCACCATCCTGACGCTGGCACCAGGTGTTTCATCCATATCTACAACACAAGGCTCCGACGGTGTT
TAGCCAGAACGAAGCGTCGCTGCTGAATCAACTGAAGAACATTGCCAATCGTGAAGATTATGTCGTGACCTG
10 GTGGGACTATGGCTACCCGGTGCGCTATTACAGCGATGTTAAACGCTGGTCGACGGCGGTAAACACCTGG
GCAAGGACAACTTTTCCCGAGCTTTGCTCTGTCTAAAGATGAACAGGCAGCTGCGAATATGGCGCGCTGT
CAGTCGAATACACCGAAAAGTCGTTTTATGCCCCGAGAATGATATTCTGAAAACGGACATCCTGCAGGCAA
TGATGAAGGATTATAACCAAAGCAATGTTGACCTGTTCTGGCGTCACTGTGAAACCGGATTTTAAGATTG
ACACCCCGAAAACGCGTGATATCTATCTGTACATGCCGGCTCGCATGAGTCTGATTTTTAGCACCGTCGCGA
15 GCTTTTCTTTCATCAACCTGGATACGGGCGTGCTGGACAAACCGTTTACCTTCTCAACGGCCTACCCGCTGG
ATGTGAAGAACGGCGAAATTTATCTGTGCAATGGTGTTCCTGAGCGATGACTTTGTTCTTTCAAATCG
GCGATAACGTTGTGAGCGTGAACAGCATCGTTGAAATTAATAGCATCAAACAGGGTGAATACAAGATTACCC
CGATCGATGACAAAGCGCAATTTTATATTTTCTACCTGAAGGACTCCGCTATTCCGTATGCGCAGTTTCATCCT
GATGGATAAAACCATGTTTAACTCTGCCTACGTTCAAATGTTTTTCTGGGTAACACTACGATAAGAACCTGTTT
20 GACCTGGTCATTAATTCTCGCGATGCAAAGGTGTTTAACTGAAGATCTAA

In certain aspects, the nucleotide sequence encoding PglB is codon optimized.

Polymerase

Host cells of the present invention may also comprise a nucleotide sequence that encodes a polymerase (*e.g. wzy*). In certain embodiments, the polymerase (*e.g. wzy*) is introduced into a host cell of the invention (*i.e.* the polymerase is heterologous to the host cell). In some embodiments, the polymerase is a bacterial polymerase. In other embodiments, the polymerase is a capsular polysaccharide polymerase (*e.g. wzy*) or an O antigen polymerase (*e.g. wzy*). In certain aspects, the polymerase is an O-antigen polysaccharide polymerase (*e.g. wzy*), *e.g.* from *Shigella* species, *Pseudomonas* species or *Escherichia* species. (*e.g. Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Pseudomonas aeruginosa*, or *E. coli*). In other aspects, the polymerase is a capsular polysaccharide polymerase (*e.g. wzy*), *e.g.* from *N. meningitidis* serogroup A (MenA), *N. meningitidis* serogroup C (MenC), *N. meningitidis* serogroup Y (MenY), *N. meningitidis* serogroup W (MenW), *H. influenzae* type b (Hib), Group B *Streptococcus* (GBS), *Streptococcus pneumoniae*, or *Staphylococcus aureus*. In yet ther apsects, the polymerase is a capsular polysaccharide polymerase (*e.g. wzy*) of *Streptococcus pneumoniae*. In some mebodiments, said *wzy* polymerase may be incorporated (*e.g.* inserted into the genome or expressed by a plasmid) in said host cell as part of a *rfb* cluster or capsular

polysaccharide cluster. Thus, in certain embodiments, a host cell of the invention may further comprise a nucleotide sequence encoding a heterologous *wzy* polymerase.

Flippases

5 A host cell of the invention may also comprise a nucleotide sequence encoding a heterologous flippase (*e.g. wzx*), *e.g.* a heterologous flippase. Flippases translocate wild type repeating units and/or their corresponding engineered (hybrid) repeat units from the cytoplasm into the periplasm of host cells (*e.g. E. coli*). In certain embodiments, the flippase is a bacterial flippase, *e.g.* a flippase of the polysaccharide biosynthetic pathway of interest. In some embodiments, the host cell of the invention
10 comprises a nucleotide sequence encoding a flippase (*e.g. wzx* gene) of a polysaccharide biosynthetic pathway of a *Streptococcus* species, *Shigella* species, *Escherichia* species, *Pseudomonas* species, or *Staphylococcus* species. (*e.g. Streptococcus pneumoniae*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *E. coli*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*. In other embodiments, the flippase is a capsular polysaccharide flippase (*e.g. wzx*) of *Streptococcus pneumoniae*. Other
15 flippases that can be introduced into the host cells of the invention are for example from *Campylobacter jejuni* (*e.g. pgkK*).

Translocases

A host cell of the invention may also comprise a nucleotide sequence encoding a translocase
20 (*e.g. wzm-wzt*), *e.g.* a heterologous translocase. In certain aspects, the translocase is a Wzx/Wzy dependent transporter. In other aspects, the translocase is a ATP-binding cassette (ABC) dependent transporter. In yet other aspects, the translocase is a synthase dependent transporter. In specific embodiments, the translocase is an ABC transporter. Polysaccharides assembled by ABC transporters are fully polymerized by sequential glycosyl transfer at the cytoplasmic face of the inner membrane
25 of a host cell. The glycan can be assembled as an Undecaprenyl diphosphate (Und-PP)-linked intermediate (*e.g.* for most O-antigen polysaccharides). Alternatively, for certain types of polysaccharides (*e.g.* capsular polysaccharides), the acceptor is diacylglycerol phosphate. The polysaccharide chain is elongated by addition of monomers to the nonreducing terminus of the lipid-linked intermediate and is completed in the cytoplasm, prior to export to the periplasmic space via the
30 translocase (*e.g.* ABC transporter) (Cuthbertson L. et al., 2010, *Microbiology and Molecular Biology Reviews*, 74(3):341-362; Bi Y. et al., 2018, *Nature*, 553(7688):361-365).

In certain embodiments, a saccharide antigen of the invention is synthesized by a ABC transporter-dependent pathway. Thus, in certain aspects, a saccharide antigen of the invention is completely synthesized on the cytosolic leaflet of the plasma membrane of a host cell (*e.g. E. coli*).
35 In certain embodiments, the saccharide antigen of the invention is built on a lipid acceptor (*e.g.* Undecaprenyl diphosphate (Und-PP)) leading to the formation of a lipid-linked antigen saccharide. In

certain aspects, Und-PP serves as a lipid acceptor and is modified by the addition of an acetylated amino sugar phosphate (e.g., N-acetylglucosamine-1-P) to generate a biosynthesis primer. In specific embodiments, a translocase (e.g. *wzm-wzt*) translocates the Und-PP-linked saccharide antigen intermediate to the membrane's periplasmic side, where it forms a substrate for glycosylation of a carrier protein (e.g. a modified Als3 protein of the invention). In certain embodiments, the translocase is a bacterial translocase, e.g. a translocase of the polysaccharide biosynthetic pathway of interest. Thus, in some embodiments, the host cell of the invention comprises a nucleotide sequence encoding a translocase (e.g. *wzm-wzt*) of a polysaccharide biosynthetic pathway of a *Klebsiella* species, *Streptococcus* species, *Shigella* species, *Escherichia* species, *Pseudomonas* species, or *Staphylococcus* species. (e.g. *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *E. coli*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*). In specific embodiments, the heterologous translocase that is introduced into a host cell of the invention is an ABC transporter (e.g. *wzm-wzt*) of *Klebsiella pneumoniae*. Other translocases that can be introduced into the host cells of the invention are for example from *Campylobacter jejuni* (e.g. *pglK*).

Thus, in certain embodiments, the present invention provides a host cell comprising a nucleotide sequence comprising (i) a *wzm* gene comprising a nucleotide sequence of SEQ ID NO: 36, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 36; and (ii) a *wzt* gene comprising a nucleotide sequence of SEQ ID NO: 37, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 37. In certain aspects, the *wzm* and *wzt* genes are from *Klebsiella pneumoniae*.

Thus, in certain aspects, the present invention provides a *wzm* gene, optionally from *Klebsiella pneumoniae*, optionally comprising a nucleotide sequence of SEQ ID NO: 36, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 36:

ATGAAGTACAATTTAGGGTATTTATTTGATTTACTTGTTGTCATAACAAATAAAGATCTAAAAGTGC
 GCTATAAGAGCAGCATGCTAGGCTATTTATGGTCAGTAGCAAATCCATTGCTTTTTGCCATGATTTATTATTT
 TATATTTAAGCTGGTAATGAGAGTACAAATTCAAATTATACCGTTTTCTCATTACCGGCTTGTTCCGTGG
 CAATGGTTTGCCAGTTCCGGCACTAACTCATTATTTTCATTCATCGCTAACGCTCAAATTATCAAGAAGACAG
 TTTTCCCCGGTCCGTGATTCCGCTAAGTAATGTAATGATGGAAGGGTTGCATTTTCTTTGTACCATCCCGGT
 TATTGTTGCTTTCTTTTGTATGGCATGACGCCGTCCTTGTCCTGGGTTTGGGGTATACCTCTCATTGCT
 ATTGGCCAGGTGATTTTACCTTTGGTGTTCATCATCTTTTCAACGCTGAACCTGTTTTCCGTGACCTGG
 AGCGCTTTGTCAGTCTGGGATTATGCTGATGTTTTATTGTACGCCGATTTTATATGCGTCTGATATGATTCC
 GGAAAAATTTAGCTGGATAATTACCTACAATCCGCTAGCGAGTATGATTCTTAGTTGGCGTGATTTATTCATG
 AATGGGACTCTTAATTATGAGTATATTTCTATACTCTATTTTACGGGAATCATTTTGACGGTTGTCGGTTTGT
 CTATTTTCAATAAAATTAATATCGATTTGCAGAGATCTTGTA

In certain aspects, the *wzm* gene encodes a Wzm protein comprising (or consisting of) an amino acid sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 18. In specific

aspects, the wzm gene encodes a Wzm protein comprising (or consisting of) an amino acid sequence of SEQ ID NO: 18:

VISAMPKGTRRTSMKYNLGYLFDLLVVITNKLKVRYSMLGYLWSVANPLLFAMIYYFIFKLVMRVQI
 PNYTVFLITGLFPWQWFASSATNSLFSFIANAQIIKKTVPFRSVIPLSNVMMEGHLFLCTIPVIVVFLFVYGMTPSL
 5 SWVWGIPLIAIGQVIFTFGVSIIFSTLNLFFRDLERFVSLGIMLMFYCTPILYASDMIPEKFSWIITYNPLASMILSW
 RDLFMNGTLNIEYISILYFTGIILTVVGLSIFNKLKYRFAEIL

Thus, additional aspects, the present invention provides a wzt gene, optionally from *Klebsiella pneumoniae*, optionally comprising a nucleotide sequence of SEQ ID NO: 37, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 37:

10 ATGCACCCAGTTATTAACCTCAGTCATGTTACAAAAGAGTATCCTCTGTACCATCATATTGGCTCAG
 GAATCAAAGATTTAATTTTCCATCCGAAACGCGCTTTTCAATTGCTGAAGGGGCGGAAATATTTAGCTATCGA
 AGACGTATCCTTTACAGTTGGCAAAGGTGAGGCTGTTGCTCTGATTGGACGTAATGGGGCAGGAAAGAGTAC
 CTCTCTGGCCTGGTTGCCGGCGTGATTAAGCCAACTAAGGGAACCGTCACCACTGAAGGACGGGTGGCATC
 GATGCTTGAACCTCGGCGGAGGCTTTCATCCGGAACCTACCGGGCGTGAGAATATTTACCTGAATGCTACTCT
 15 GCTGGGCCTTCGGCGTAAAGAGGTCCAGCAACGTATGGAACGTATTATTGAATTTTCGGAACCTGGGAGAATT
 CATAGACGAGCCAATCAGAGTGTACTCAAGCGGAATGCTAGCTAAGTTAGGTTTTTCGGTCATCAGTCAGGT
 TGAACCGGATATTTTAATTATTGATGAAGTTCTGGCAGTAGGTGATATCGCTTTTCAGGCAAAATGTATTAG
 ACCATAAGAGATTTTAAGAAAAGAGGCGTGACAATATTGTTTGTAGCCACAATATGAGTGACGTTGAAAAA
 ATCTGCGACAGATCATCTGGATCGAAAATCATAGGCTCAGAGAAGTGGGGTCTGCAGAGCGAATCATTGAA
 20 CTGTACAAGCAAGCAATGGCTTAA

In some aspects, the wzt gene encodes a Wzt protein comprising (or consisting of) an amino acid sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 19. In specific aspects, the wzt gene encodes a Wzt protein comprising (or consisting of) an amino acid sequence of SEQ ID NO: 19:

25 MHPVINFSHVTKEYPLYHHIGSGIKDLIFHPKRAFQLLKGRKYLAIEDVSFTVGKGEAVALIGRNGAGKS
 TSLGLVAGVIKPTKGTVTTEGRVASMLELGGGFHPELTGRENIYLNATLLGLRRKEVQQRMERIIEFSELGEFIDE
 PIRVYSSGMLAKLGFVSISQVEPDILIIDEVLAVGDIAFQAKCIQTIRDFKKRGVTILFVSHNMDSVEKICDRVIWIE
 NHRLREVGSAERIIELYKQAMA

30 In additional embodiments, the present invention provides a method of producing a β -1,3 glucan polymer in a host cell of the invention, the method comprising the steps of introducing and expressing in the host cell:

- i. a nucleotide sequence encoding a first glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule;

- 5
- ii.

a nucleotide sequence encoding additional glycosyltransferases capable of synthesizing a fungal β -1, 3 glucan, wherein the additional glycosyltransferases comprise SleC, SleE, SleF, SleU and SleW from rhizobia; and
- iii.

optionally, a nucleotide sequence encoding a translocase capable of translocating the β -1, 3 glucan to periplasmic side of an inner membrane of the host cell,

wherein the β -1,3 glucan polymer is linked to a lipid carrier via the GlcNAc.

In specific aspects, the present invention provides a method of producing a β -1,3 glucan polymer in a prokaryotic host cell, the method comprising the steps of introducing and expressing in the host cell:

- 10
- i.

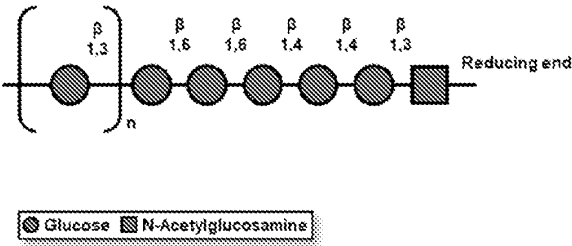
a nucleotide sequence encoding a first glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule, wherein the first glycosyltransferase is WfaP from *E. coli* O56;
- ii.

a nucleotide sequence encoding additional glycosyltransferases capable of synthesizing a fungal β -1, 3 glucan, wherein the additional glycosyltransferases comprise SleC, SleE, SleF, SleU and SleW from rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09, and wherein the host cell produces more SleW than SleC, SleE, SleF or SleU; and
- 15
- iii.

optionally, a nucleotide sequence encoding a translocase capable of translocating the β -1, 3 glucan to periplasmic side of an inner membrane of the prokaryotic host cell, wherein the translocase comprises Wzm-Wzt from *Klebsiella* sp., optionally from *Klebsiella pneumoniae*,
- 20

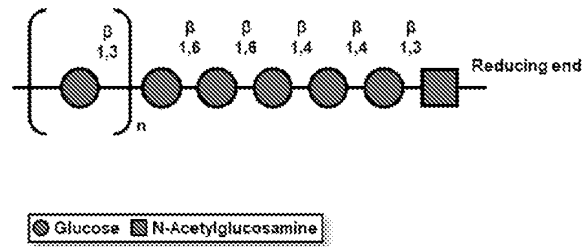
wherein the β -1,3 glucan polymer is linked to a lipid carrier via the GlcNAc and wherein the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules.

In certain aspects, the β -1,3 glucan polymer is a fungal β -1,3 glucan polymer. In some aspects, the fungal β -1,3 glucan polymer is of *Candida*. In specific aspects, the fungal β -1,3 glucan polymer is of *Candida albicans*. In certain embodiments, the β -1,3 glucan polymer has the structure:



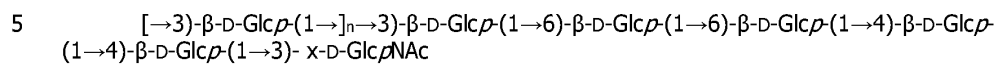
wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

In other embodiments, the β -1,3 glucan polymer has the structure:



wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35 or 6-25.

In additional embodiments, the β -1,3 glucan polymer has the structure:



wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

In some aspects, the prokaryotic host cell is a bacterial cell. In some aspects, the bacteria is a gram positive bacteria. In other aspects, the bacteria is a gram negative bacteria. In certain embodiments, the bacteria includes, but is not limited to, an *Escherichia* species, a *Shigella* species, *Klebsiella* species, a *Xanthomonas* species, a *Salmonella* species, a *Yersinia* species, a *Lactococcus* species, a *Lactobacillus* species, a *Pseudomonas* species, a *Corynebacterium* species, a *Streptomyces* species, a *Streptococcus* species, a *Staphylococcus* species, a *Bacillus* species, and a *Clostridium* species. In some aspects, the bacteria is an *E. coli* species. In specific aspects, the bacteria is *E. coli*. In certain embodiments, the prokaryotic host cell includes, but is not limited to, an *Escherichia* species, a *Shigella* species, *Klebsiella* species, a *Xanthomonas* species, a *Salmonella* species, a *Yersinia* species, a *Lactococcus* species, a *Lactobacillus* species, a *Pseudomonas* species, a *Corynebacterium* species, a *Streptomyces* species, a *Streptococcus* species, a *Staphylococcus* species, a *Bacillus* species, and a *Clostridium* species. In some aspects, the prokaryotic host cell is an *E. coli* species. In specific embodiments, the prokaryotic host cell is *E. coli*. In certain embodiments, the GlcNAc is linked to a lipid carrier by WecA from the host *E. coli* cell. In specific embodiments, the lipid carrier is undecaprenyl.

Bioconjugates

25 In certain embodiments, the present invention provides a bioconjugate comprising a modified Als3 protein of the invention linked to an antigen, as described herein. In certain aspects, the antigen is linked to an amino acid on the modified Als3 protein selected from asparagine, aspartic acid, glutamic acid, lysine, cysteine, tyrosine, histidine, arginine or tryptophan (*e.g.* asparagine). Bioconjugates, as described herein, have advantageous properties over chemical conjugates of antigen-carrier protein, in that they require less chemicals in manufacture and are more consistent in terms of the final product generated.

In certain embodiments, the present invention provides a method of producing a bioconjugate that comprises a modified Als3 protein of the invention linked to at least one saccharide antigen, the method comprising: (1) culturing a host cell of the invention under conditions suitable for the production of proteins; and (2) isolating the bioconjugate produced by a host cell of the invention. In some embodiments, the bioconjugate is isolated or purified from a whole cell extract of the host cell. In other embodiments, the bioconjugate is isolated or purified from a cytoplasmic extract from the host cell. In specific embodiments, the bioconjugate is isolated or purified from a periplasmic extract from the host cell.

Methods of making Bioconjugates are known in the art. For example, Bioconjugates of the invention can be made using the shakeflask process, *e.g.* in a LB shake flask. In certain aspects, a fed-batch process for the production of recombinant glycosylated proteins in bacteria can be used to produce bioconjugates of the invention. The aim is to increase glycosylation efficiency and recombinant protein yield per cell and while maintaining simplicity and reproducibility in the process. Bioconjugates of the invention can be manufactured on a commercial scale by developing an optimized manufacturing method using typical *E. coli* production processes. Various types of feed strategies, such as batch, chemostat and fed-batch can be used.

Thus, in specific embodiments, the present invention provides a bioconjugate produced by a method of the invention. In specific aspects, a bioconjugate of the invention comprises at least one saccharide antigen linked to a modified Als3 protein of the invention.

The bioconjugates of the invention can be purified for example, by chromatography (*e.g.* ion exchange, anionic exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. See, *e.g.* Saraswat *et al.* 2013, Biomed. Res. Int. ID#312709 (p. 1-18); see also the methods described in WO 2009/104074. Further, the bioconjugates of the invention may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

In additional embodiments, the present invention provides a method of producing a glycoconjugate comprising a modified carrier protein and at least one saccharide antigen. As used herein, the term "glycoconjugate" refers to a hybrid molecule composed of a carrier protein and multiple polysaccharide chains, wherein the polysaccharides are covalently linked to the carrier protein.

In certain aspects, the modified carrier protein of the invention includes, but is not limited to, Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural

glycoprotein. In specific aspects, the modified carrier protein is a modified Als3 protein of the invention.

In certain embodiments, the at least one saccharide antigen comprises a β -1,3 glucan polymer. In some aspects, the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules. In additional aspects, the β -1,3 glucan polymer comprises four to hundred, four to fifty, four to forty, four to thirty five, four to thirty, four to twenty five, four to twenty, four to ten, four to nine, four to eight, four to seven, four to six, four to five, four, five to hundred, five to fifty, five to forty, five to thirty five, five to thirty, five to twenty five, five to twenty, five to ten, five to nine, five to eight, five to seven, five to six, five, six to hundred, six to fifty, six to forty, six to thirty five, six to thirty, six to twenty five, six to twenty, six to ten, six to nine, six to eight, six to seven, six, seven to hundred, seven to fifty, seven to forty, seven to thirty five, seven to thirty, seven to twenty five, seven to twenty, seven to ten, seven to nine, seven to eight, or seven β -1,3 linked glucose molecules. In some aspects, the β -1,3 glucan polymer comprises at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 11 consecutive β -1,3 linked glucose molecules. In specific embodiments, the β -1,3 glucan polymer comprises at least 11 β -1,3 linked glucose molecules. In particular embodiments, the β -1,3 glucan polymer comprises at least 11 consecutive β -1,3 linked glucose molecules.

In certain embodiments, the present invention provides a method of producing a glycoconjugate comprising a modified carrier protein and a β -1,3 glucan. In some embodiments, the method of producing a glycoconjugate comprising a modified carrier protein and a β -1,3 glucan comprises culturing the host cell the invention under conditions suitable for the production of proteins.

In further embodiments, the present invention provides a method of producing a bioconjugate in a host cell of the invention, the method comprising the steps of:

- i. obtaining a host cell of the invention that produces a β -1,3 glucan polymer; and
- ii. further introducing and expressing in the host cell:
 - a. a nucleotide sequence encoding a modified carrier protein comprising at least one glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline, and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner membrane of the host cell; and
 - b. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein.

In certain aspects, the present invention provides a method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:

- a. obtaining a prokaryotic host cell of the invention that produces a β -1,3 glucan polymer;
and
- 5 b. further introducing and expressing in the host cell:
 - i. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline, and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of
10 transporting the modified carrier protein to the periplasmic side of the inner membrane of the prokaryotic host cell; and
 - ii. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase
15 is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli*.

In additional aspects, the present invention provides a method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:

- c. obtaining a prokaryotic host cell of the invention that produces a β -1,3 glucan polymer;
20 and
- d. further introducing and expressing in the host cell:
 - iii. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline (optionally a polynucleotide sequence
25 of the invention), and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner membrane of the prokaryotic host cell; and

- a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a
30 bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli*.

In certain aspects, the modified carrier protein of the invention includes, but is not limited to, Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid,

detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural glycoprotein. In specific aspects, the modified carrier protein is a modified Als3 protein of the invention.

In certain embodiments, the bacterial signal sequence is selected from, without limitation, FlgI, MalE, OmpA, and OmpC. In specific aspects, the bacterial signal sequence is FlgI. In certain aspects, the FlgI comprises an amino acid sequence of SEQ ID NO: 21. In particular embodiments, the bacterial signal sequence is removed from the modified carrier protein after the modified carrier protein is transported to the periplasmic side of the inner membrane of the prokaryotic host cell.

Analytical Methods

Various methods can be used to analyze the structural compositions and sugar chain lengths of the bioconjugates of the invention and to determine glycosylation site usage.

Hydrazinolysis can be used to analyze glycans. First, polysaccharides are released from their protein carriers by incubation with hydrazine according to the manufacturer's instructions (Ludger Liberate Hydrazinolysis Glycan Release Kit, Oxfordshire, UK). The nucleophile hydrazine attacks the glycosidic bond between the polysaccharide and the carrier protein and allows release of the attached glycans. N-acetyl groups are lost during this treatment and have to be reconstituted by re-N-acetylation. The free glycans are purified on carbon columns and subsequently labeled at the reducing end with the fluorophor 2-amino benzamide. See Bigge JC, Patel TP, Bruce JA, Goulding PN, Charles SM, Parekh RB, Anal Biochem 1995, 230(2):229-238. The labeled polysaccharides are separated on a GlycoSep-N column (GL Sciences) according to the HPLC protocol of Royle et al.. See Royle L, Mattu TS, Hart E, Langridge JI, Merry AH, Murphy N, Harvey DJ, Dwek RA, Rudd PM, Anal Biochem 2002, 304(1):70-90. The resulting fluorescence chromatogram indicates the polysaccharide length and number of repeating units. Structural information can be gathered by collecting individual peaks and subsequently performing MS/MS analysis. Thereby the monosaccharide composition and sequence of the repeating unit can be confirmed and additionally in homogeneity of the polysaccharide composition can be identified. Alternatively, high mass MS and size exclusion HPLC can be applied to measure the size of the complete bioconjugates.

Yield may be measured as carbohydrate amount derived from a liter of bacterial production culture grown in a bioreactor under controlled and optimized conditions. After purification of bioconjugate, the carbohydrate yields can be directly measured by either the anthrone assay or ELISA using carbohydrate specific antisera. Indirect measurements are possible by using the protein amount (measured by BCA, Lowry, or bardford assays) and the glycan length and structure to calculate a

theoretical carbohydrate amount per gram of protein. In addition, yield can also be measured by drying the glycoprotein preparation from a volatile buffer and using a balance to measure the weight.

Various methods can be used to analyze the conjugates of the invention including, for example, SDS-PAGE or capillary gel electrophoresis. Polymer length is defined by the number of repeat units that are linearly assembled. This means that the typical ladder like pattern is a consequence of different repeat unit numbers that compose the glycan. Thus, two bands next to each other in SDS PAGE (or other techniques that separate by size) differ by only a single repeat unit. These discrete differences are exploited when analyzing glycoproteins for glycan size: the unglycosylated carrier protein and the bioconjugate with different polymer chain lengths separate according to their electrophoretic mobilities. The first detectable repeat unit number (n_1) and the average repeat unit number (n_{average}) present on a bioconjugate are measured. These parameters can be used to demonstrate batch to batch consistency or polysaccharide stability, for example.

Glycosylation site usage may be quantified by, for example, glycopeptide LC-MS/MS: conjugates are digested with protease(s), and the peptides are separated by a suitable chromatographic method (C18, Hydrophilic interaction HPLC HILIC, GlycoSepN columns, SE HPLC, AE HPLC), and the different peptides are identified using MS/MS. This method can be used with or without previous sugar chain shortening by chemical (smith degradation) or enzymatic methods. Quantification of glycopeptide peaks using UV detection at 215 to 280nm allows relative determination of glycosylation site usage. In other embodiments, site usage may be quantified by size exclusion HPLC: Higher glycosylation site usage is reflected by an earlier elution time from a SE HPLC column. In yet other embodiments, site usage may be quantified by quantitative densitometry of purified bioconjugates stained with Coomassie Brilliant Blue following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

25 **Immunogenic Compositions and Vaccines**

The conjugates (*e.g.* bioconjugate), of the invention are particularly suited for inclusion in immunogenic compositions and vaccines.

Thus, in certain embodiments, the present invention provides an immunogenic composition comprising a conjugate or a bioconjugate of the invention. In certain aspects, the immunogenic composition additionally comprises a pharmaceutically acceptable excipient and/or carrier. In specific aspects, the present invention provides an immunogenic composition comprising a modified Als3 protein of the invention, a conjugate of the invention, or a bioconjugate of the invention. In other aspects, the immunogenic composition additionally comprises a pharmaceutically acceptable excipient and/or carrier.

Immunogenic compositions comprise an immunologically effective amount of a modified Als3 protein of the invention, or conjugate (*e.g.* bioconjugate) of the invention, as well as any other

components. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either as a single dose or as part of a series is effective for treatment or prevention. This amount varies depending on the health and physical condition of the individual to be treated, age, the degree of protection desired, the formulation of the vaccine and other relevant factors.

5 Pharmaceutically acceptable excipients and carriers are described, for example, in Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co. Easton, PA, 5th Edition (1975). Pharmaceutically acceptable excipients can include a buffer, such as a phosphate buffer (*e.g.* sodium phosphate). Pharmaceutically acceptable excipients can include a salt, for example sodium chloride. Pharmaceutically acceptable excipients can include a solubilizing/stabilizing agent, for
10 example, polysorbate (*e.g.* TWEEN 80). Pharmaceutically acceptable excipients can include a preservative, for example 2-phenoxyethanol or thiomersal. Pharmaceutically acceptable excipients can include a carrier such as water or saline.

In additional embodiments, the present invention provides a method of making the immunogenic composition of the invention comprising the step of mixing a modified Als3 protein of
15 the invention, a conjugate of the invention, or a bioconjugate of the invention, with a pharmaceutically acceptable excipient or carrier.

Preferably, an immunogenic composition of the invention is formulated as a vaccine for in vivo administration to a subject (*e.g.* human) wherein the individual components of the composition are formulated such that the immunogenicity of individual components is not impaired by other individual
20 components of the composition (see above definition). Thus, in some embodiments, no (significantly) detrimental effect occurs to the saccharide antigen (in terms of protective efficacy) in the combination as compared to their administration in isolation. Preferably, an immunogenic composition of the invention is formulated as a vaccine for in vivo administration to a subject (*e.g.* human), which confers an antibody titre superior to the criterion for seroprotection for each antigenic component for an
25 acceptable percentage of human subjects.

Thus, in additional embodiments, the present invention provides a vaccine comprising an immunogenic composition of the invention. In certain embodiments, the vaccine additionally comprises a pharmaceutically acceptable excipient or carrier. In other embodiments, the vaccine additionally comprises an adjuvant. In specific embodiments, the present invention provides a vaccine
30 comprising an immunogenic composition of the invention and, optionally, a pharmaceutically acceptable excipient or carrier.

In specific embodiments, the present invention provides a *Candida albicans* vaccine comprising: (1) a modified Als3 protein of the invention; (2) at least one *Candida albicans* saccharide antigen linked to said modified Als3 protein; and, optionally, (3) a pharmaceutically acceptable carrier
35 or adjuvant.

The term "adjuvant" refers to a compound that when administered in conjunction with or as part of an immunogenic composition of vaccine of the invention augments, enhances and/or boosts the immune response to modified Als3 protein conjugate/bioconjugate, but when the compound is administered alone does not generate an immune response to the modified Als3 protein conjugate/bioconjugate. Adjuvants can enhance an immune response by several mechanisms including, *e.g.* lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. Specific examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see United Kingdom Patent GB2220211), MF59 (Novartis), AS01 (GlaxoSmithKline), and saponins, such as QS21 (see Kensil *et al.* in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund's adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute *et al.* N. Engl. J. Med. 336, 86-91 (1997)). A suitable adjuvant is an adjuvant comprising an oil in water emulsion, wherein said oil in water emulsion comprises a metabolisable oil, a tocol and an emulsifier. Suitably, the metabolisable oil is squalene, the tocol is alpha-tocopherol and the emulsifying agent is polyoxyethylene sorbitan monooleate.

In some embodiments, the adjuvant includes an oil-in-water emulsion. For example, the oil-in-water emulsion can include an oil phase that incorporates a metabolisable oil, and an additional oil phase component, such as a tocol. The oil-in-water emulsion may also contain an aqueous component, such as a buffered saline solution (*e.g.*, phosphate buffered saline). In addition, the oil-in-water emulsion typically contains an emulsifier. In some embodiments, the metabolizable oil is squalene. In certain embodiments, the tocol is alpha-tocopherol. In some embodiments, the emulsifier is a nonionic surfactant emulsifier (such as polyoxyethethylene sorbitan monooleate, TWEEN80™). In exemplary embodiments, the oil-in-water emulsion contains squalene and alpha tocopherol in a ratio which is equal or less than 1 (w/w).

In certain embodiments, the metabolisable oil in the oil-in-water emulsion may be present in an amount of 0.5-10mg. In some embodiments, the tocol in the oil-in-water emulsion may be present in an amount of 0.5 – 11 mg. In some embodiments, the emulsifying agent may be present in an amount of 0.4 – 4 mg.

In order for any oil in water composition to be suitable for human administration, the oil phase of the emulsion system has to comprise a metabolisable oil. The meaning of the term metabolisable oil is well known in the art. Metabolisable can be defined as 'being capable of being transformed by metabolism' (Dorland's Illustrated Medical Dictionary, W.B. Sanders Company, 25th edition (1974)). The oil may be any vegetable oil, fish oil, animal oil or synthetic oil, which is not toxic to the recipient and is capable of being transformed by metabolism. Nuts, seeds, and grains are common sources of

vegetable oils. Synthetic oils are also part of this invention and can include commercially available oils such as NEOBEE® (caprylic/capric triglycerides made using glycerol from vegetable oil sources and medium-chain fatty acids (MCTs) from coconut or palm kernel oils) and others. A particularly suitable metabolisable oil is squalene. Squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene) is an unsaturated oil which is found in large quantities in shark-liver oil, and in lower quantities in olive oil, wheat germ oil, rice bran oil, and yeast, and is a particularly preferred oil for use in this invention. Squalene is a metabolisable oil by virtue of the fact that it is an intermediate in the biosynthesis of cholesterol (Merck index, 10th Edition, entry no.8619).

Tocols are well known in the art and are described in EP0382271. Suitably the tocol is alpha-tocopherol or a derivative thereof such as alpha-tocopherol succinate (also known as vitamin E succinate). In certain embodiments, said tocol is suitably present in an amount of 0.5-11 mg.

The oil in water emulsion further comprises an emulsifying agent. The emulsifying agent may suitably be polyoxyethylene sorbitan monooleate. In a particular embodiment the emulsifying agent may be Polysorbate® 80 (Polyoxyethylene (20) sorbitan monooleate) or Tween® 80. In certain embodiments, said emulsifying agent is suitably present in the adjuvant composition in an amount of 0.4-4mg.

Also provided is a method of making the immunogenic composition of the invention comprising the step of mixing the modified Als3 protein or the conjugate (*e.g.* bioconjugate) of the invention with a pharmaceutically acceptable excipient and/or carrier and an adjuvant. Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds Powell M.F. & Newman M.J.) (1995) Plenum Press New York).

The immunogenic compositions of the invention can be included in a container, pack, or dispenser together with instructions for administration. The immunogenic compositions or vaccines of the invention can be stored before use, *e.g.* the compositions can be stored frozen (*e.g.* at about -20°C or at about -70°C); stored in refrigerated conditions (*e.g.* at about 4°C); or stored at room temperature. The immunogenic compositions or vaccines of the invention may be stored in solution or lyophilized. In certain embodiments, the solution is lyophilized in the presence of a sugar such as sucrose, trehalose or lactose. In additional embodiments, the vaccines of the invention are lyophilized and extemporaneously reconstituted prior to use.

30

Administration and Dosage

Immunogenic compositions or vaccines of the invention may be used to protect or treat a subject (*e.g.* human), by means of administering said immunogenic composition or vaccine via systemic or mucosal route. These administrations may include injection via the intramuscular (IM), intraperitoneal, intradermal (ID) or subcutaneous (SC) routes; or via mucosal administration to the oral/alimentary, respiratory, genitourinary tracts.

35

In certain aspects, the immunogenic composition or vaccine of the invention is administered by the intramuscular delivery route. Intramuscular administration may be to the thigh or the upper arm. Injection is typically via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

5 In another aspects, the immunogenic composition or vaccine of the invention is administered by the intradermal administration. Human skin comprises an outer "horny" cuticle, called the stratum corneum, which overlays the epidermis. Underneath this epidermis is a layer called the dermis, which in turn overlays the subcutaneous tissue. The conventional technique of intradermal injection, the "mantoux procedure", comprises steps of cleaning the skin, and then stretching with one hand, and
10 with the bevel of a narrow gauge needle (26 to 31 gauge) facing upwards the needle is inserted at an angle of between 10 to 15. Once the bevel of the needle is inserted, the barrel of the needle is lowered and further advanced whilst providing a slight pressure to elevate it under the skin. The liquid is then injected very slowly thereby forming a bleb or bump on the skin surface, followed by slow withdrawal of the needle.

15 In additional aspects, the immunogenic composition or vaccine of the invention is administered by the intranasal administration. Typically, the immunogenic composition or vaccine is administered locally to the nasopharyngeal area, *e.g.* without being inhaled into the lungs. It is desirable to use an intranasal delivery device which delivers the immunogenic composition or vaccine formulation to the nasopharyngeal area, without or substantially without it entering the lungs. Suitable devices for
20 intranasal administration of the vaccines according to the invention are spray devices. Suitable commercially available nasal spray devices include ACCUSPRAY™ (Becton Dickinson).

The amount of conjugate (*e.g.* bioconjugate) in each immunogenic composition or vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific
25 immunogen is employed and how it is presented. The content of a conjugate (*e.g.* a bioconjugate) will typically be in the range 1-100µg, suitably 5-50µg for the sachcharide dose, *e.g.*, glucan dose.

Prophylactic and Therapeutic Uses

The present invention also provides an immunogenic composition of the invention, or the
30 vaccine of the invention, for use in medicine. The use of an immunogenic composition of the invention in the manufacture of a medicament for the treatment or prevention of diseases caused by infection by *C. albicans* is also envisioned, as is a method of immunising a subject (*e.g.* a human) against disease caused by *C. albicans*, which method comprises administering to the subject an immunoprotective dose of an immunogenic composition of the invention.

35 In certain embodiments, the present invention provides a method of inducing immune response to a fungal infection in a subject (*e.g.* human), the method comprising administering to the

subject a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention. In certain embodiments, the modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention can be used to induce an immune response against fungi. In certain aspects, the fungi is *Candida* species. In some aspects, the *Candida* species includes, but is not limited to, *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanaea* and *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*. In specific embodiments, the *Candida* species is *Candida albicans*. In certain embodiments, said subject has fungal infection at the time of administration. In other embodiments, said subject does not have a fungal infection at the time of administration.

In specific embodiments, the present invention provides a method of inducing immune response to *Candida albicans* infection in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.

The present invention also provides methods of treating and/or preventing a fungal infection in a subject comprising administering to the subject a conjugate (*e.g.* bioconjugate) of the invention. The conjugate (*e.g.* bioconjugate) may be in the form of an immunogenic composition or vaccine. Thus, in certain embodiments, the present invention provides a method for treatment or prevention of fungal infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention. The conjugate (*e.g.* bioconjugate) may be in the form of an immunogenic composition or vaccine. In other embodiments, the present invention provides a method for immunizing a subject against fungal infection, the method comprising administering to the subject an immunoprotective dose of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of any of the invention, an immunogenic composition of the invention, or a vaccine of the invention. In still other embodiments, the present invention provides a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention for use in the manufacture of a medicament for the treatment or prevention of a disease caused by fungal infection in a subject (*e.g.* human). In certain aspects, the fungi is *Candida* species. In some aspects, the *Candida* species includes, but is not limited to, *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanaea* and *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*. In specific embodiments, the *Candida* species is *Candida albicans*.

This, in specific embodiments, the present invention provides a method for treatment or prevention of *Candida albicans* infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition
5 of the invention, or a vaccine of the invention.

In other specific embodiments, the present invention provides a method for immunizing a subject against *Candida albicans* infection, the method comprising administering to the subject an immunoprotective dose of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of any of the invention, an immunogenic composition of the invention, or a vaccine of
10 the invention.

In specific embodiments, the present invention provides a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention for use in the manufacture of a medicament for the treatment or prevention of a disease caused by *Candida albicans* infection in a subject (*e.g.* human).
15

In other embodiments, the present invention provides a method of inhibiting adhesion of *Candida albicans* hyphae to vaginal epithelial cells in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.
20

In further embodiments, the present invention provides a method of mediating neutrophil killing of *Candida albicans* hyphae in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.
25

In additional embodiments, the present invention provides a method of inhibiting biofilm formation of *Candida albicans*, the method comprising: (i) administering to a subject (*e.g.* human) a therapeutically or prophylactically effective amount of a modified Als3 protein of the invention, a conjugate of the invention, a bioconjugate of the invention, an immunogenic composition of the invention, or a vaccine of the invention.
30

Embodiments of the invention are further described in the subsequent numbered paragraphs:

1. A modified Agglutinin-like sequence 3 (Als3) protein comprising amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%,
35 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, modified in that the

amino acid sequence comprises one or more consensus sequences comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

2. The modified Als3 protein of paragraph 1, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids selected from the group consisting of one or more amino acids between amino acid residues 18-23, one or more amino acids between amino acid residues 28-42, one or more amino acids between amino acid residues 75-87, one or more amino acids between amino acid residues 82-92, one or more amino acids between amino acid residues 99-113, one or more amino acids between amino acid residues 114-126, one or more amino acids between amino acid residues 118-132, one or more amino acids between amino acid residues 150-164, one or more amino acids between amino acid residues 158-169, one or more amino acids between amino acid residues 163-177 (*e.g.* one or more amino acids between amino acid residues 168-172), one or more amino acids between amino acid residues 170-184 (*e.g.* one or more amino acids between amino acid residues 175-179), one or more amino acids between amino acid residues 202-212, one or more amino acids between amino acid residues 215-225, one or more amino acids between amino acid residues 231-242, one or more amino acids between amino acid residues 265-275, one or more amino acids between amino acid residues 271-281, one or more amino acids between amino acid residues 281-292, and one or more amino acids between amino acid residues 294-305 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
3. The modified Als3 protein of any of paragraphs 1 and 2, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 18 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
4. The modified Als3 protein of any of paragraphs 1 to 3, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 33-37 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

5. The modified Als3 protein of any of paragraphs 1 to 4, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 80-82 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
6. The modified Als3 protein of any of paragraphs 1 to 5, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 87 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
7. The modified Als3 protein of any of paragraphs 1 to 6, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
8. The modified Als3 protein of any of paragraphs 1 to 7, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 119-121 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
9. The modified Als3 protein of any of paragraphs 1 to 8, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 123-127 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
10. The modified Als3 protein of any of paragraphs 1 to 9, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 155-159 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

11. The modified Als3 protein of any of paragraphs 1 to 10, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 163-164 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
12. The modified Als3 protein of any of paragraphs 1 to 11, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 207 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
13. The modified Als3 protein of any of paragraphs 1 to 12, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 220 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
14. The modified Als3 protein of any of paragraphs 1 to 13, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 236-237 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
15. The modified Als3 protein of any of paragraphs 1 to 14, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 270 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
16. The modified Als3 protein of any of paragraphs 1 to 15, wherein the one or more consensus sequences have been added next to or substituted for amino acid residue 276 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

17. The modified Als3 protein of any of paragraphs 1 to 16, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 286-287 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
18. The modified Als3 protein of any of paragraphs 1 to 17, wherein the one or more consensus sequences have been added next to or substituted for one or more amino acids between amino acid residues 299-300 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
19. The modified Als3 protein of any of paragraphs 1 and 2, wherein the one or more consensus sequences have been substituted for (i) the amino acids between amino acid residues 33-37 and (ii) the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
20. The modified Als3 protein of any of paragraphs 1 to 19, wherein substitution of the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1 results in an increase in expression level of the modified Als3 protein relative to a control Als3 protein.
21. The modified Als3 protein of paragraph 20, wherein the expression level of the modified Als3 protein is increased at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold relative to the control Als3 protein.
22. The modified Als3 protein of any of paragraphs 20 and 21, wherein the expression level of the modified Als3 protein is increased about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, or about 10-fold relative to the control Als3 protein.

23. The modified Als3 protein of any of paragraphs 1 to 22, wherein the modified Als3 protein is of *Candida*.
24. The modified Als3 protein of paragraph 23, wherein the *Candida* is selected from the group consisting of *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanaea* and *Candida tropicalis*.
25. The modified Als3 protein of any of paragraph 23 and 24, wherein the modified Als3 protein is of *Candida albicans*.
26. The modified Als3 protein of any of paragraphs 1 to 25, wherein the modified Als3 protein further comprises at least one Fructose biphosphate aldolase-1 (Fba) peptide comprising an amino acid sequence of YGKDVKDLFDYAE (SEQ ID NO: 3) or an amino acid sequence at least 70%, 80%, 85%, 90%, or 92% identical to SEQ ID NO: 3.
27. The modified Als3 protein of paragraph 26, wherein the Fba peptide is covalently linked to the modified Als3 protein at one or more amino acid residues selected from the group consisting of 89, 163, 259, 199 and 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent position(s) within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
28. The modified Als3 protein of any of paragraphs 26 and 27, wherein the Fba peptide is covalently linked to the modified Als3 protein at amino acid residue 316 of amino acid residues 18-316 of SEQ ID NO: 1 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
29. The modified Als3 protein of any of paragraphs 26 to 28, wherein the modified Als3 protein comprises at least one additional consensus sequence comprising an amino acid sequence of J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise 1 to 5 naturally occurring amino acid residues, wherein the at least one consensus sequence has been added next to C-terminal amino acid residue of SEQ ID NO: 3 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 3.

30. The modified Als3 protein of paragraph 29, wherein X is Q, Z is A, each of J and B comprises 1 to 5 Glycine (G) residues and U comprises 1 to 5 serine (S) residues.
31. The modified Als3 protein of any of paragraphs 29 and 30, wherein the additional consensus sequence comprises an amino acid sequence of GSGGGDQNATGSGGG (SEQ ID NO: 9).
- 5 32. The modified Als3 protein of any of paragraphs 1 to 31, wherein at least one of the one or more consensus sequences comprises an amino acid sequence of K-D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.
33. The modified Als3 protein of paragraph 32, wherein X is Q (glutamine), Z is A (alanine) and the one or more consensus sequences are selected from the group consisting of KDQNAT
10 (SEQ ID NO: 5), KDQNAS (SEQ ID NO: 6) and DQNAT (SEQ ID NO: 7).
34. A modified Als3 protein comprising an amino acid sequence of SEQ ID NO: 10.
35. A modified Als3 protein comprising an amino acid sequence of SEQ ID NO: 11.
36. The modified Als3 protein of any of paragraphs 1 to 35, wherein the modified Als3 protein is glycosylated.
- 15 37. The modified Als3 protein of any of paragraphs 1 to 36, wherein the modified Als3 protein is N-glycosylated.
38. A conjugate comprising the modified Als3 protein of any of paragraphs 1 to 35 and at least one saccharide antigen.
39. The conjugate of paragraph 38, wherein the modified Als3 protein is linked to the at least one
20 saccharide antigen.
40. The conjugate of any of paragraphs 38 and 39, wherein the at least one saccharide antigen is selected from the group consisting of: O antigens of *E. coli*, *Salmonella* sp. O antigens, *Pseudomonas* sp., *Klebsiella* sp. O antigens, *Acinetobacter* O antigens, *Chlamydia trachomatis* O antigens, *Vibrio cholera* O antigens, *Listeria* sp. O antigens, *Legionella pneumophila* serotypes 1 to 15 O antigens, *Bordetella parapertussis* O antigens, *Burkholderia mallei* and
25 *pseudomallei* O antigens, *Francisella tularensis* O antigens, *Campylobacter* sp. O antigens, capsular polysaccharides of *Clostridium difficile*, *Staphylococcus aureus* type 5 and 8,

5 *Streptococcus pyrogenes*, *E. coli*, *Streptococcus agalacticae*, *Neisseria meningitidis*, *Candida* sp., *Candida albicans*, *Haemophilus influenza*, *Enterococcus faecalis* capsular polysaccharides type I-V, and other surface polysaccharide structures, e.g. the *Borrelia burgdorferi* glycolipids, *Neisseria meningitidis* pilin O glycan and lipooligosaccharide (LOS), *Haemophilus influenza* LOS, *Leishmania major* lipophosphoglycan, tumor associated carbohydrate antigens, malaria glycosyl phosphatidylinositol, and *mycobacterium tuberculosis* arabinomannan.

41. The conjugate of any of paragraphs 38 and 39, wherein the at least one saccharide antigen is a fungal saccharide antigen.
- 10 42. The conjugate of any of paragraphs 38 to 41, wherein the at least one saccharide antigen is a saccharide antigen of *Candida* species.
43. The conjugate of paragraph 42, wherein the *Candida* species is selected from the group consisting of *Candida albicans*, *Candida auris*, *Candida guilliermondii*, *Candida lusitanae* and *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*.
- 15 44. The conjugate of paragraph any of paragraphs 38 to 43, wherein the at least one saccharide antigen is a saccharide antigen of *Candida albicans*.
45. The conjugate of any of paragraphs 38 to 44, wherein the at least one saccharide antigen comprises a β -1,3 glucan polymer.
46. The conjugate of paragraph 45, wherein the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules.
- 20 47. The conjugate of any of paragraphs 45 and 46, wherein the β -1,3 glucan polymer comprises four to hundred, four to fifty, four to forty, four to thirty five, four to thirty, four to twenty five, four to twenty, four to ten, four to nine, four to eight, four to seven, four to six, four to five, four, five to hundred, five to fifty, five to forty, five to thirty five, five to thirty, five to twenty five, five to twenty, five to ten, five to nine, five to eight, five to seven, five to six, five, six to hundred, six to fifty, six to forty, six to thirty five, six to thirty, six to twenty five, six to twenty, six to ten, six to nine, six to eight, six to seven, six, seven to hundred, seven to fifty, seven to forty, seven to thirty five, seven to thirty, seven to twenty five, seven to twenty, seven to ten, seven to nine, seven to eight, or seven β -1,3 linked glucose molecules.
- 25

48. The conjugate of any of paragraphs 45 to 47, wherein the β -1,3 glucan polymer comprises at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 11 consecutive β -1,3 linked glucose molecules.
- 5 49. The conjugate of any of paragraphs 38 to 44, wherein the at least one saccharide antigen comprises a β -1,2 mannan polymer.
50. The conjugate of paragraph 48, wherein the β -1,2 mannan polymer comprises at least two β -1,2 linked mannose molecules.
- 10 51. The conjugate of any of paragraphs 49 and 50, wherein the β -1,2 mannan polymer comprises two to fifty, two to forty, two to thirty, two to twenty, two to ten, two to nine, two to eight, two to seven, two to six, two to five, two to four, two to three, two, three to fifty, three to forty, three to thirty, three to twenty, three to ten, three to nine, three to eight, three to seven, three to six, three to five, three to four, three, four to fifty, four to forty, four to thirty, four to twenty, four to ten, four to nine, four to eight, four to seven, four to six, four to five, four, five to fifty, five to forty, five to thirty, five to twenty, five to ten, five to nine, five to eight, five to seven, five to six, or five, six to forty, six to thirty, six to twenty, six to ten, six to nine, six to eight, six to seven, six, seven to fifty, seven to forty, seven to thirty, seven to twenty, seven to ten, seven to nine, seven to eight, or seven 1,2 linked mannose molecules.
- 15 52. The conjugate of any of paragraphs 49 to 51, wherein the β -1,2 mannan polymer comprises at least 2, at least 3, at least 4, or at least 5 consecutive β -1,2 linked mannose molecules.
- 20 53. The conjugate of any of paragraphs 38 to 52, wherein the modified Als3 protein is linked to the at least one saccharide antigen at one or more amino acid residues on the modified Als3 protein, wherein the one or more residues are selected from the group consisting of one or more asparagine residues, one or more aspartic acid residues, one or more glutamic acid residues, one or more lysine residues, one or more cysteine residues, one or more tyrosine residues, one or more histidine residues, one or more arginine residues, one or more tryptophan residues, one or more serine residues, and one or more threonine residues.
- 25 54. The conjugate of any of paragraphs 38 to 53, wherein the modified Als3 protein is linked to the at least one saccharide antigen at one or more asparagine residues on the modified Als3 protein.

55. The conjugate of any of paragraphs 38 to 54, wherein the conjugate is a bioconjugate.
56. A modified Als3 protein of *Candida albicans* consisting of: (1) an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11; and (2) at least one saccharide antigen of *Candida*, wherein the at least one saccharide antigen is a β -1,3 glucan polymer consisting of at least six consecutive β -1,3 linked glucose molecules, and wherein the at least one saccharide antigen is linked to at least one of three asparagine residues at positions 20, 92, and 324 of SEQ ID NO: 10 or positions 20, 92, and 337 of SEQ ID NO: 11.
57. A polynucleotide sequence encoding the modified Als3 protein of any of paragraphs 1 to 35.
58. A vector comprising the polynucleotide sequence of paragraph 57.
59. A host cell comprising: (1) one or more polynucleotide sequences that encode one or more heterologous glycosyltransferases; (2) a polynucleotide sequence that encodes a heterologous oligosaccharyl transferase; (3) a polynucleotide sequence that encodes a modified Als3 protein according to any of paragraphs 1 to 35; and, optionally, (4) a polynucleotide sequence that encodes a polymerase.
60. The host cell of paragraph 59, wherein the host cell is *Escherichia coli*.
61. A method of producing a bioconjugate that comprises a modified Als3 protein linked to at least one saccharide antigen, the method comprising: (1) culturing the host cell of any of paragraphs 59 and 60 under conditions suitable for the production of proteins; and (2) isolating the bioconjugate.
62. A bioconjugate produced by the method of paragraph 61, wherein said bioconjugate comprises at least one saccharide linked to a modified Als3 protein of any of paragraphs 1 to 35.
63. An immunogenic composition comprising the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 54, or the bioconjugate of any of paragraphs 55 and 62.
64. A method of making the immunogenic composition of paragraph 63, the method comprising the step of mixing the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 54, or the bioconjugate any of paragraphs 55 and 62, with a pharmaceutically acceptable excipient or carrier.

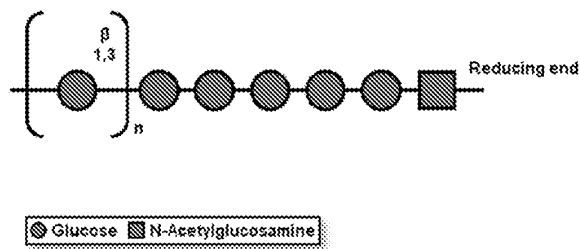
65. A vaccine comprising the immunogenic composition of paragraph 63 and, optionally, a pharmaceutically acceptable excipient or carrier.
- 5 66. A *Candida albicans* vaccine comprising: (1) the modified Als3 protein of any of paragraphs 1 to 35; (2) at least one *Candida albicans* saccharide antigen linked to said modified Als3 protein; and, optionally, (3) a pharmaceutically acceptable carrier or adjuvant.
- 10 67. A method for treatment or prevention of *Candida albicans* infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the modified Als3 protein of any of paragraph 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
68. The method of paragraph 54, wherein the *Candida albicans* infection causes Recurrent Vulvovaginal Candidiasis (RVVC) in the subject.
- 15 69. A method for immunizing a subject against *Candida albicans* infection, the method comprising administering to the subject an immunoprotective dose of the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
- 20 70. A method for inducing immune response to *Candida albicans* infection in a subject, the method comprising administering to the subject a therapeutically or prophylactically effective amount of the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
71. The method of any of paragraphs 66 to 70, wherein the subject is a human.
- 25 72. The modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66 for use in treatment or prevention of a disease caused by *Candida albicans* infection.

- 5 73. The modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66, for use in the manufacture of a medicament for the treatment or prevention of a disease caused by *Candida albicans* infection.
- 10 74. A method for increasing expression level of the modified Als3 protein of any of paragraphs 1 to 37, the method comprising substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein exhibits an increased expression level relative to a control Als3 protein which does not comprise one or more consensus sequences substituted for amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.
- 15 75. The method of paragraph 74, wherein the expression level of the modified Als3 protein is increased at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold relative to the control Als3 protein.
- 20 76. The method of paragraph 75, wherein the expression level of the modified Als3 protein is increased about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, or about 10-fold relative to the control Als3 protein.
- 25 77. A host cell comprising:
- i. a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;
 - ii. A nucleotide sequence encoding a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule;
 - iii. a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and
 - iv. optionally, a nucleotide sequence encoding a modified carrier protein comprising
- 30 a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

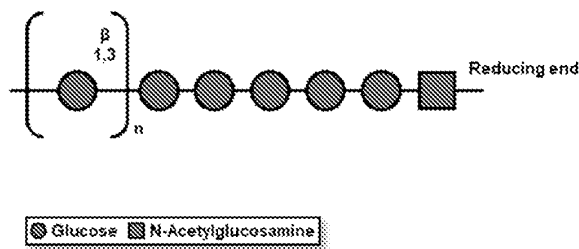
78. The host cell of paragraph 77, wherein the one or more heterologous glycosyltransferases of i. comprises exoL, exoM, exoO, exoU and exoW from a rhizobia, optionally from *Sinorhizobium*, optionally from *Sinorhizobium meliloti* 1021.
- 5 79. The host cell of paragraph 77, wherein the one or more heterologous glycosyltransferase(s) of i. comprises SleC, SleE, SleF, SleU and SleW from a rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09.
80. The host cell of paragraph 79, wherein the one or more heterologous glycosyltransferase of i. has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleC of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 12.
- 10 81. The host cell of any of paragraphs 79 and 80, wherein the one or more heterologous glycosyltransferase(s) of i. has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleE of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 13.
- 15 82. The host cell of any one of paragraphs 79 to 81, wherein the one or more heterologous glycosyltransferase(s) of i. has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleF of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 14.
- 20 83. The host cell of any one of paragraphs 79 to 82, wherein the one or more heterologous glycosyltransferase(s) of i. has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleU of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 15.
- 25 84. The host cell of any one of paragraphs 79 to 83, wherein the one or more heterologous glycosyltransferase(s) of i. has an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of SleW of *Agrobacterium* sp. ZX09 comprising SEQ ID NO: 16.
85. The host cell of any one of paragraphs 79 to 84, wherein the host cell produces more SleW than SleC, SleE, SleF or SleU.

86. The host cell of any one of paragraphs 79 to 85, wherein the host cell contains at least two gene copies of a SleW of *Agrobacterium* sp. ZX09.
87. The host cell of any of paragraphs 79 and 86, wherein the host cell contains at least one copy of a SleW of *Agrobacterium* sp. ZX09 under the control of a strong promoter that enables higher expression of SleW compared to SleC, SleE, SleF or SelU.
88. The host cell of any one of paragraphs 77 to 87, wherein the glycosyltransferase capable of covalently bonding a glucose to GlcNac of ii. is WfaP from *E. coli* 056.
89. The host cell of paragraph 88, wherein the WfaP comprises an amino acid sequence at least 80%, 90%, 95%, 98%, or 99% identical to SEQ ID NO: 17.
90. The host cell of any one of claims 77 to 89 comprising a nucleotide sequence comprising (i) a wzm gene comprising a nucleotide sequence of SEQ ID NO: 36, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 36; and (ii) a wzt gene comprising a nucleotide sequence of SEQ ID NO: 37, optionally comprising a nucleotide sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO: 37.
91. The host cell of any one of paragraphs 77 to 90, wherein the oligosaccharyl transferase of iii. is a PglB.
92. The host cell of paragraph 91, wherein the PglB is from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli* comprising an amino acid sequence of SEQ ID NO: 20.
93. The host cell of any one of paragraphs 77 to 92, wherein the modified carrier protein of iv. is selected from the group consisting of Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural glycoprotein.
94. The host cell of paragraph 93, wherein the modified carrier protein is the modified Als3 protein of any one of paragraphs 1 to 35.

95. The host cell of any one of paragraphs 77 to 94, wherein said host cell is an *Escherichia* species, *Shigella* species, *Klebsiella* species, *Xhantomonas* species, *Salmonella* species, *Yersinia* species, *Lactococcus* species, *Lactobacillus* species, *Pseudomonas* species, *Corynebacterium* species, *Streptomyces* species, *Streptococcus* species, *Staphylococcus* species, *Bacillus* species, or a *Clostridium* species.
96. The host cell of paragraph 95, wherein said host cell is an *E. coli* species.
97. A method of producing a glycoconjugate comprising a modified carrier protein and a β -1,3 glucan, wherein said method comprises culturing the host cell of any one of paragraphs 77 to 96 under conditions suitable for the production of proteins.
98. A bioconjugate produced by the method of paragraph 97.
99. A saccharide which is a glucan having the structure:

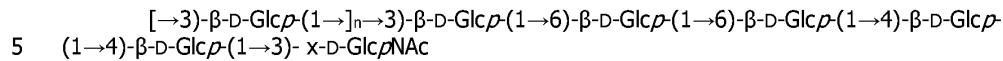


- wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.
100. The saccharide of paragraph 99 having the structure:



wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35 or 6-25.

101. A saccharide which is a glucan having the structure:



wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

102. The saccharide of any one of paragraphs 99-101 which is linked to a lipid carrier.

103. The saccharide of paragraph 102, wherein the lipid carrier is undecaprenyl.

10 104. A conjugate comprising the saccharide of any one of paragraphs 99-101 linked to an asparagine residue of a modified carrier protein.

105. The conjugate of paragraph 104, wherein the asparagine residue is within a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

15 106. The conjugate of any of paragraphs 104 and 105, wherein the modified carrier protein is selected from the group consisting of Als3, Sap2, detoxified Exotoxin A of *P. aeruginosa* (EPA), CRM197, Diphtheria toxoid, tetanus toxoid, detoxified hemolysin A of *S. aureus*, clumping factor A of *S. aureus*, clumping factor B of *S. aureus*, *E. coli* FimH, *E. coli* FimHC, *E. coli* heat labile enterotoxin, detoxified variants of *E. coli* heat labile enterotoxin, Cholera toxin B subunit (CTB), cholera toxin, detoxified variants of cholera toxin, *E. coli* sat protein, the passenger domain of *E. coli* sat protein, *C. jejuni* AcrA, and a *C. jejuni* natural glycoprotein.

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107. The conjugate of any one of paragraphs 104 to 106, wherein the carrier protein is the modified Als3 protein of any one of paragraphs 1 to 35.

108. The conjugate of any one of paragraphs 104 to 107, wherein the conjugate is a bioconjugate.

25 109. A method of producing a β -1,3 glucan polymer in a prokaryotic host cell, the method comprising the steps of introducing and expressing in the host cell:

- 5 i. a nucleotide sequence encoding a first glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule, wherein the first glycosyltransferase is WfaP from *E. coli* O56;
- ii. a nucleotide sequence encoding additional glycosyltransferases capable of synthesizing a fungal β -1, 3 glucan, wherein the additional glycosyltransferases comprise SleC, SleE, SleF, SleU and SleW from rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09, and wherein the host cell produces more SleW than SleC, SleE, SleF or SleU; and
- 10 iii. optionally, a nucleotide sequence encoding a translocase capable of translocating the β -1, 3 glucan to periplasmic side of an inner membrane of the prokaryotic host cell, wherein the translocase comprises Wzm-Wzt from *Klebsiella* sp., optionally from *Klebsiella pneumoniae*,
- wherein the β -1,3 glucan polymer is linked to a lipid carrier via the GlcNAc and wherein the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules.
- 15 110. The method of paragraph 109, wherein the prokaryotic host cell is *E. coli*.
111. The method of paragraph 110, wherein the GlcNAc is linked to a lipid carrier by WecA from the host *E. coli* cell.
112. The method of any of paragraphs 109 to 111, wherein the β -1,3 glucan polymer is a fungal β -1,3 glucan polymer, optionally of *Candida*, optionally of *Candida albicans*.
- 20 113. The method of any one of paragraphs 109-112 wherein the β -1, 3 glucan polymer has the structure of the saccharide of any one of paragraphs 99-101.
114. The method of any of paragraphs 109 to 113, wherein the lipid carrier is undecaprenyl.
115. A method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:
- 25 a. obtaining a prokaryotic host cell of any of paragraphs 109 to 114 that produces a β -1,3 glucan polymer; and
- b. further introducing and expressing in the host cell:

- 5 i. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline, and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner membrane of the prokaryotic host cell; and
- 10 ii. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli*.
116. The method of paragraph 115, wherein the bacterial signal sequence is selected from the group consisting of: FlgI, MalE, OmpA, and OmpC.
117. The method of paragraph 116, wherein the bacterial signal sequence is FlgI.
- 15 118. The method of paragraph 116, wherein the FlgI comprises an amino acid sequence of SEQ ID NO: 21.
119. The method of any of paragraphs 115 to 118, wherein the bacterial signal sequence is removed from the modified carrier protein after the modified carrier protein is transported to the periplasmic side of the inner membrane of the prokaryotic host cell.
- 20 120. The method of any of paragraphs 115 to 119, wherein the modified carrier protein is the modified Als3 protein of any of paragraphs 1 to 35.
- 25 121. A method for increasing expression level of the modified Als3 protein of any of paragraphs 1 to 37 in a host cell, the method comprising substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein when expressed in a host cell exhibits an increased expression level relative to a control Als3 protein which does not comprise one or more consensus sequences substituted for amino acids between amino acid

residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

122. A method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:

a. obtaining a prokaryotic host cell of any of paragraphs 109 to 114 that produces a β -1,3 glucan polymer; and

b. further introducing and expressing in the host cell:

i. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline (optionally a polynucleotide sequence of paragraph 57), and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner membrane of the prokaryotic host cell; and

ii. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or *Campylobacter coli*.

123. The modified Als3 protein of any of paragraphs 26 to 28, wherein the modified Als3 protein comprises at least one additional consensus sequence comprising an amino acid sequence of J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise 1 to 5 naturally occurring amino acid residues, wherein the at least one additional consensus sequence has been added next to C-terminal amino acid residue of SEQ ID NO: 3 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 3.

124. A host cell comprising: (1) one or more polynucleotide sequences that encode one or more heterologous glycosyltransferases; (2) a polynucleotide sequence that encodes a

glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNac) molecule; (3) a polynucleotide sequence that encodes a heterologous oligosaccharyl transferase; (4) a polynucleotide sequence that encodes a modified Als3 protein according to any of paragraphs 1 to 35; and, optionally, a polynucleotide sequence that encodes a polymerase.

- 5
125. A host cell comprising:
- i. a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;
 - 10 ii. A nucleotide sequence encoding a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNac) molecule;
 - 15 iii. a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and optionally, a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline, optionally a polynucleotide sequence of paragraph 57.
126. A method of inhibiting adhesion of *Candida albicans* hyphae to vaginal epithelial cells in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
- 20
127. A method of mediating neutrophil killing of *Candida albicans* hyphae in a subject (*e.g.* human), the method comprising administering to the subject a therapeutically or prophylactically effective amount of the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
- 25
128. A method of inhibiting biofilm formation of *Candida albicans*, the method comprising: (i) administering to a subject (*e.g.* human) a therapeutically or prophylactically effective amount of the modified Als3 protein of any of paragraphs 1 to 37 and 56, the conjugate of any of paragraphs 38 to 55, the bioconjugate of any of paragraphs 55 and 62, the immunogenic composition of paragraph 63, or the vaccine of any of paragraphs 65 and 66.
- 30

All publications mentioned herein are herein incorporated by reference in their entirety. It is to be understood that the term "or," as used herein, denotes alternatives that may, where appropriate, be combined; that is, the term "or" includes each listed alternative separately as well as their combination. As used here, unless the context clearly dictates otherwise, references to the singular, such as singular forms "a," "an," and "the," include the plural, and references to the plural include the singular.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

10

EXAMPLES

Material and methods

Engineering of Als3-NT for glycosylation with antigenic glycans

15 In order to predict suitable positions for insertion of glycosites, the crystal structure of an Als3 protein of the invention having an amino acid sequence of SEQ ID NO: 27 ("Als3-NT") was analyzed using various software.

SEQ ID NO: 27 - Als3-NT (amino acids 18-316 of SEQ ID NO: 1) sequence of *Candida albicans*:

20 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTASPGDTFTLNMPGVKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNLTPTSIKALGTVTLPALFNVGGTGSSVDLEDSKCFTAGTNTVTFNDDGG
KKISINVDFERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSESFSYTKTCCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRPFTLRWT
GYR

25 20 positions in total were selected for insertion of the consensus sequence for glycosylation i.e., glycosite (*e.g.* D/E-X-N-Z-S/T) by site directed mutagenesis (Table 1 and FIG. 1). These positions included the N- and C-termini of the Als3-NT domain as well as its solvent accessible loops and some beta-strands. One or more (up to 5) amino acids were substituted by the glycosite sequence to create a "modified Als3-NT protein." In some cases, the glycosite was inserted in between two amino acids
30 of the Als3-NT protein without creating any sequence substitution. Modified Als3-NT proteins comprising single glycosites were tested for glycosylation with *Klebsiella pneumoniae* O5 antigen. The best performing glycosites were combined to generate modified Als3-NT proteins comprising between two to six glycosites in total. The six positions that were selected for combinations were: 33-37 (Mut1), 104-108 (Mut4), 163-164 (Mut8), 220 (Mut12), 299-300 (Mut18), and 316 (C-terminus),
35 where the numbering corresponds to the residues of the native *Candida albicans* Als3 sequence (SEQ ID NO: 1).

Glycosylation tests with engineered Als3-NT comprising one or more glycosites

Modified Als3-NT proteins comprising a single inserted glycosite were tested for *in vivo* glycosylation efficiency using *Klebsiella pneumoniae* O5 antigen. For the data set presented in this work, the used *E. coli* W3110-derivative strain included the deletion of the LPS-O antigen ligase waaL and contained the cluster of genes for the biosynthesis of *Klebsiella pneumoniae* O5 glycan replacing the native O antigen cluster rfbO16. The *E. coli* strain producing KpO5 glycan was transformed with a pEC415 plasmid carrying a modified Als3-NT protein and a plasmid expressing PglB. To prepare a pre-culture, 5 ml TB medium comprising 10 mM MgCl₂ and appropriate antibiotics was inoculated with a streak of colonies from the transformation plate and grown at 37°C o/n. The pre-culture was used to inoculate 50 ml of supplemented TB medium in a shake flask to give a starting OD₆₀₀ = 0.1. The cultures were grown at 37°C, with 200 rpm shaking until reaching OD₆₀₀ = 0.8-1 and then induced by addition of 0.001% arabinose (Als3) and 0.1 mM IPTG (PglB). The expression and glycosylation of modified Als3-NT proteins was continued at 25°C overnight.

The selection criteria for modified Als3-NT proteins with single glycosite included the total expression level and the level of produced glycoconjugate, the later indicating suitability of glycosite position for modification by PglB.

Periplasmic extract preparation

The amount of cells from overnight cultures corresponding to OD₆₀₀ = 60 (measured using a spectrophotometer) was harvested by centrifugation. The cell pellets were resuspended in 1.5 ml of lysis buffer (30 mM Tris-HCl pH 8.5, 1 mM EDTA (Ethylenediaminetetraacetic acid), 20% sucrose) and lysozyme was added to a final concentration of 1 mg/ml. The suspensions were incubated with slight shaking for 25 minutes at 4°C and then centrifuged at 16,000 rcf for 10 min. After centrifugation, the supernatant corresponding to periplasmic extract (PPE) was transferred to a fresh tube.

Enrichment of periplasmic extract by immobilized metal affinity chromatography (IMAC)

In order to enrich periplasmic extracts with modified Als3-NT proteins and allow more direct read-out by SDS-PAGE, His-tagged modified Als3-NT proteins were purified using one-step purification on Ni-NTA (Nickel Nitrilo-triacetic Acid) agarose. 1ml of PPE was mixed with 200 µl of pre-equilibrated Ni-NTA slurry and incubated with slight shaking for 30 min. After that the resin was washed and the bound protein eluted with elution buffer (30 mM Tris pH 8.0, 500 mM imidazole, 50 mM NaCl). The IMAC enriched PPE was analysed by SDS-PAGE. "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4", *Nature*, 227 (5259): 680–685. Bibcode:1970Natur.227..680L. doi:10.1038/227680a0. ISSN 0028-0836. PMID 5432063). Unglycosylated Als3-NT proteins and glycoconjugates (*i.e.* modified Als3-NT proteins linked to one or more polysaccharide chains)

glycosylated at one or more positions were detected on the gel by Coomassie staining (Fazekas de St. Groth, S.; Webster, R. G.; Dwyer, A. (1963). "Two new staining procedures for quantitative estimation of proteins on electrophoretic strips". *Biochimica et Biophysica Acta*. 71: 377–391. doi:10.1016/0006-3002(63)91092-8. PMID 18421828).

5

Western blot analysis of periplasmic extract

Periplasmic extracts were also analysed by immunoblots against the His-tag and against polysaccharide attached to the modified Als3-NT proteins. For detection of KpO5, anti-serum against K-capsular mutant of *Klebsiella pneumoniae* O5 strain was used.

10

Construction of the Modified Als3-NT- β -1,3-glucan-producing strain

An *E. coli* K12 W3110-derivative strain was constructed by subsequent replacements of the targeted gene clusters with the gene of interest, if any, followed by an FRT sites-flanked selection marker via λ -Red homologous recombination followed by FLP recombinase-catalysed marker removal as described (TE Kuhlman and EC Cox. *Nucleic Acids Res.* 2010 Apr; 38(6): e92). Five homologous recombination / marker removal steps were carried out, removing genomic sequences of i. O16 O-antigen cluster (*rfb* or *wb*, GenBank NC_007779 position 2'114'113 to 2'103'814), ii. colanic acid cluster (*wca*, GenBank NC_007779 position 2'138'241 to 2'118'033), iii. ECA cluster retaining *wecA* (*wec*, GenBank NC_007779 position 3'666'604 to 3'656'725), iv. O16wzz2 or *clb* (GenBank NC_007779 position 2'099'458 to 2'100'438), v. *gtrABS* or *yfdGHI* (GenBank NC_007779 position 2'473'301 to 2'475'908), vi. *araBA* (GenBank NC_007779 position 66'835 to 70'048). Moreover, a codon-optimized version of *C. jejuni* *pglB* (GenBank WP_002866139) was introduced, replacing the LPS-O antigen ligase *walL* (GenBank NC_007779 position 3'842'208 to 3'843'467).

An expression plasmid comprising the gene for the selected modified Als3-NT protein, followed by the genes necessary for the β -1,3-glucan biosynthesis in a pEC415 backbone (Schulz et al. *J Biol Chem.* 1998 Aug; 281(5380):1197-200) was constructed in different steps of classical restriction cloning and Gibson assembly (Gibson, D.G., et al. (2009) *Nat. Methods* 6, 343-345) starting from synthetic DNA templates. The final plasmid contains the following genes under arabinose-inducible promoter, from 5' to 3': als3-NT, *sleW*, *sleU*, *sleF*, *sleE*, *sleC*, *wfaP*, *wzm*, and *wzt*. Modified Als3-NT protein is described above; *sleW*, *sleU*, *sleF*, *sleE*, *sleC* sequences are from *Agrobacterium* sp. ZX09 succinoglucon-like exopolysaccharide biosynthesis gene cluster (GenBank KT780309; Xu et al. *Appl Microbiol Biotechnol.* (2017) 101:585–598); *wfaP* sequence is from *E. coli* O56 (GenBank DQ220293); *wzm* and *wzt* are from *K. pneumoniae* (GenBank CP052562 position 1'695'622 to 1'697'129).

A second expression plasmid was constructed by cloning a codon-optimized version of *C. jejuni* *pglB* (GenBank WP_002866139) into the vector pEXT21 (Dykxhoorn et al. *Gene*. 1996. 177(1-2):133-6) under IPTG-inducible promoter.

35

The conjugate-producing strain was obtained by transforming the engineered strain with the two plasmids. The role of each gene in the production of the bioconjugate, as well as the structure of the glycan are schematized in FIG. 2.

5 *Production and analysis of the Modified Als3-NT-β-1,3-glucan bioconjugate*

 The conjugate-producing strain was grown in a fed-batch 10-L bioreactor in a buffered rich medium at 35°C and pH 7. When OD_{600nm} of the culture registered a value of 20-25, the temperature was switched to 30°C, the bioconjugate production was induced with IPTG and arabinose, and a rich medium feed was started. Cells were harvested by centrifugation 18 hours after induction and washed
10 and resuspended in TBSE buffer. An osmotic shock protocol was applied in which the cells are diluted in a 5-fold volume of H₂O for 1 hour, realising the content of the periplasm in the supernatant. Cells were separated from the supernatant by centrifugation, and cell debris were removed by filtration through 0.45 and 0.2 μm filters. The purification of the bioconjugate from the filtered supernatant consisted in 4 chromatography steps: i. anion exchange, ii. anion exchange, iii. hydrophobic
15 interaction, iv. size exclusion. The elution profiles were followed with absorbance at 280 nm and SDS-PAGE/Coomassie staining. The purified bioconjugate was analysed by SDS-PAGE gels followed by i. coomassie staining, ii. anti-Als3 immunoblot, iii. anti-Fba immunoblot, iv. anti-β-1,3-glucan immunoblot, and dectin-1 blot, as reported in FIG. 5.

20 The results of these experiments are shown in FIGS. 3 to 16 and as described in the following Examples.

Example 1: SDS PAGE analysis of Modified Als3-NT protein-glycosite variants purified from PPE by IMAC

25 SDS-PAGE analysis (FIG. 3) was carried out on IMAC enriched periplasmic extract of *E.coli* strains producing KpO5 polysaccharide and expressing PglB and modified Als3-NT proteins with a glycosite D/E-X-N-Z-S/T introduced at the following positions into SEQ ID NO: 1:

Table 1:

Modified Protein Name	SEQ ID NO.:	Residues in Als3-NT replaced/modified by glycosite	Lane (Fig. 3)
Als3_18-316-N	43	18	3
Als3_18-316-C	44	316	4

Als3_18-316- Mut1	45	33-37	5
Als3_18-316- Mut2	46	80-82	6
Als3_18-316- Mut3	47	87	7
Als3_18-316- Mut4	48	104-108	8
Als3_18-316- Mut5	49	119-121	9
Als3_18-316- Mut6	50	123-127	10
Als3_18-316- Mut7	51	155-159	11
Als3_18-316- Mut8	52	insert between 163-164	12
Als3_18-316- Mut9	53	168-172	13
Als3_18-316- Mut10	54	175-179	14
Als3_18-316- Mut11	55	207	15
Als3_18-316- Mut12	56	220	16
Als3_18-316- Mut13	57	insert between 236-237	17
Als3_18-316- Mut14	58	270	18
Als3_18-316- Mut15	59	276	19
Als3_18-316- Mut17	61	286-287	20
Als3_18-316- Mut18	62	insert between 299-300	21

The bands shown in FIG. 3 correspond to the unglycosylated modified Als3-NT protein ("u carrier"), and to KpO5-modified Als3-NT bioconjugates ("conjugate") with one occupied glycosite.

Conclusion:

5 As shown in FIGS. 3 and 4, 19 out of 20 modified Als3-NT proteins with single glycosite are expressed to a comparable or higher level than wild type (wt) Als3-NT protein (SEQ ID NO: 27). Surprisingly, the Mut4 variant has a >2-fold increase in expression compared to wt Als3-NT expression. FIG. 4A shows the relative expression level of the modified Als3-NT proteins comprising a single glycosite compared to expression level of wt Als3-NT. FIG. 4B shows the glycosylation efficiency of
10 the modified Als3-NT proteins comprising a single glycosite.

Glycosylation of modified Als3-NT protein with KpO5 at 17 of the 19 positions was confirmed to be equally good or superior compared to unglycosylated wt Als3-NT control (FIG. 3, lane 2). In particular, Als3-NT variants with a glycosite D/E-X-N-Z-S/T introduced at positions 33-37 (Mut1), 104-108 (Mut4), 163-164 (Mut8), 220 (Mut12), 299-300 (Mut18), or 316 (C-terminal glycotag) look
15 superior compared to unglycosylated wt Als3-NT control. The modified Als3-NT proteins used in this analysis comprised a histine tag, however a skilled person will recognize that modified Als3-NT proteins with the histidine tag removed could also be used for the analysis. Thus, in certain embodiments, the invention provides for modified Als3-NT proteins with the histidine tag removed.

20 Example 2: Western Blot analysis of purified modified Als3-NT protein-glucan conjugate

Immunoblot analysis was carried out on periplasmic extract of *E.coli* strains producing KpO5 antigen polysaccharide and expressing PglB and modified Als3-NT proteins with 1 glycosite introduced at the positions shown in Table 1 above. FIG. 5A shows SDS-PAGE analysis of purified unglycosylated Als3-NT-Fba (SEQ ID NO: 10) (lane 1) and purified Als3-NT-glucan conjugate (lane 2). M represents
25 the protein standard. FIGS. 5B-5D represent immunoblots probed with anti-Als3 antibody (FIG. 5B), anti-Fba antibody (FIG. 5C), and anti-glucan antibody (FIG. 5D), respectively. FIG. 5E represents recognition of the glucan conjugated to a modified Als3-NT protein by Dectin-1 receptor. The bands in FIGS. 5B-5E correspond to the unglycosylated wt Als3-NT protein (lane 1) and to a modified Als3-NT protein-glucan bioconjugate with three occupied glycosites (lane 2).

30 Conclusion:

The SDS-PAGE shows that a modified Als3-NT protein-glucan conjugate could be purified to high purity and that increase in molecular weight compared to unglycosylated Als3-NT-Fba indicates significant glycosylation level with beta-glucan. The four independent Western blot analyses prove antigen identity, since specific antibodies against Als3, Fba peptide and β -1,3-glucan have been used.
35 The Western blot in FIG.5E shows that the glucan chains comprise at least eleven β -1,3 linked

glucoses, since the recognition by Dectin-1 receptor requires this minimal β -1,3-glucan length (Palma et al., 2006, *J Biol Chem.*, 281(9): 5771–9).

Example 3: Assays to show functionality of modified Als3-NT-SPR with fibronectin

5 The adhesive function of Als3 is within the N-terminal (NT) region that carries peptide binding cavity to which various peptidic ligands can bind (Lin, J et al, J. Biol. Chem. 2014; 2. Coleman, DA et al, J. Mol. Meth. 2009; 3. Schmidt, CS A et al, Vaccine 2012). The aim was to show that the modified Als3-NT proteins expressed in *E.coli* periplasm had structure and function that was preserved and identical as in its native source *Candida albicans*. An additional aim was to confirm
10 that Als3-NT glycosite engineering and glycosylation did not impact the protein function.

Assay set up:

Surface Plasma Resonance (SPR) assays for testing binding of a modified Als3-NT protein to its natural ligand fibronectin based on a modification of a published protocol (Ielasi et al. 2016, mBio 7(4): e00584-16). Modified Als3-NT proteins were captured on NTA chip via C-terminal His₁₀ tag (SEQ
15 ID NO: 72). Fibronectin was added to the mobile phase and tested for binding at 8 concentrations (from 450 nM to 3.5 nM). Interaction between modified Als3-NT proteins and Fibronectin was analysed by multi-cycle kinetics. 1:1 binding mode was applied for kinetics fitting. SPR sensograms and the measured parameters are shown in FIG. 6.

Conclusion:

20 As shown in FIG. 6, an engineered unglycosylated modified Als3-NT protein ("uAls³¹⁸⁻³¹⁶-3S;" middle panel) or a glycosylated modified Als3-NT protein ("β-glucan-Als³¹⁸⁻³¹⁶-3S;" right panel) shows very similar binding to fibronectin as unmodified wt Als3-NT protein ("Als³¹⁸⁻³¹⁶wt;" left panel) with K_D values of 264, 272 and 280 nM, respectively. Therefore, the structure and function of Als3 is preserved upon engineering and glycosylation.

25

Example 4: Immunogenicity of modified Als3-NT protein-glucan bioconjugate

FIG. 7 shows the preclinical testing of modified Als3-NT protein-glucan bioconjugate (Als3-NT-3S-Fba_bgluc^{d+}; Als3-3FG) in rabbit. FIG. 7A shows the bioconjugate attributes. FIG. 7B shows a 3D
30 representation of a modified Als3-NT protein-glucan bioconjugate. Structure of a modified Als3-NT protein is shown as cartoon. Spheres represent positions of the three introduced glycosites. The conjugated beta-glucan chain is schematically represented, the position and the sequence of the Fba peptide sequence is shown in red. FIG. 7C shows the rabbit immunization scheme with the modified Als3-NT protein-glucan bioconjugate.

35 FIGS. 8A and 8B show the immunogenicity of modified Als3-NT protein-glucan bioconjugate in rabbit. Modified Als3-NT protein-glucan bioconjugate: Als³¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates

buffer immunized animals, all groups tested with AS03. New Zealand White (NZW) rabbits immunized three times on a two-week interval. Coating ELISA: 8A) modified Als3 not engineered with His-Tag and 8B) Fba peptide. Als3 or Fba-specific serum IgG concentration (arbitrary units, AU) in pre-, post-II, post-III (d0, d28, d42) rabbit sera by treatment group. Lines indicate Geometric Mean Concentration (GMC) +/-95% confidence interval. ****: $p < 0.0001$, one-way ANOVA. Ref: 36_010.

Conclusion:

- 1) Modified Als3-NT protein-glucan bioconjugate is immunogenic. Significant titer increase pre/post-II and pre/post-III shown (FIG. 8A).
- 2) Fba peptide (part of the modified Als3-NT protein-glucan bioconjugate) is immunogenic. Significant titer increase pre/post-II and pre/post-III shown (FIG. 8B).

Example 5: Immunogenicity of modified Als3-NT protein-glucan bioconjugate (glycan)

FIG. 9 shows the immunogenicity of modified Als3-NT protein-glucan bioconjugate in rabbit. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval. Coating ELISA: β -glucan extract. β -glucan specific serum IgG concentration (arbitrary units, AU) in pre-, post-II, post-III (d0, d28, d42) rabbit sera by treatment group. Lines indicate GMC +/-95% confidence interval. ****: $p < 0.0001$, one-way ANOVA.

Conclusion:

- As shown in FIG. 9, the modified Als3-NT protein-glucan bioconjugate comprising β -glucan is immunogenic. Significant titer increase pre/post-II and pre/post-III shown.

Example 6: Quantitative adhesion inhibition assay to plastic

FIG. 10 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit adhesion of *C. albicans* hyphae to plastic. Sera was mixed with *C. albicans* hyphae (ATCC90028), added to plastic wells and incubated for 4hs. After wash, viable cells were measured using CellTiter Glo®. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval. Graph depicts Mean+SD. ****: $p < 0.0001$, one-way ANOVA.

Conclusion:

- As shown in FIG. 10, sera against modified Als3-NT protein-glucan bioconjugate comprising β -glucan inhibits adhesion of *C. albicans* hyphae to plastic. Significant reduction of adhesion to plastic wells is shown pre/post-III.

Example 7: Quatitative adhesion assay of *C. albicans* hyphae

FIGS. 11A and 11B show the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit adhesion of *C. albicans* to vaginal epithelial cells. FIG.11A: adhesion quantification; FIG 11B: microscopy image of *Candida* adhered to epithelial cells. Sera was mixed with *C. albicans* (SC5314), added to epithelial cells (A431) and incubated for 1.5hs. After wash, adhered cells were measured using Concavalin A – Alexa fluor 488. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval. Graph depicts Mean+SD. ****; p<0.0001, one-way ANOVA.

10 Conclusion:

As shown in FIGS. 11A and 11B, sera against modified Als3-NT protein-glucan bioconjugate inhibits adhesion of *C. albicans* hyphae to vaginal epithelial cells. Significant reduction of adhesion to epithelial cells is shown compared to Control sera at post-III.

15 Example 8: Antibody binding to *C. albicans* hyphae

FIGS. 12 and 13 show the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to bind to *C. albicans* cells. FIG. 12 shows antibody binding to *C. albicans* hyphae using whole cell ELISA. Coating ELISA: *C. albicans* hyphae (SC5314) clinical isolate grown in RPMI.

FIG.13 shows microscopy image of antibodies bound to *C. albicans* cells. Cells were observed with differential interference contrast microscopy (DIC) and fluorescent microscopy (secondary antibody Alexa 488). Sera was mixed with *C. albicans* (SC5314) and coloured using Concavalin A – Alexa fluor 488. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval.

25 Conclusion:

As shown in FIG. 12 and in FIG. 13, antibodies against modified Als3-NT protein-glucan bioconjugate are able to bind *C. albicans* cells coated on plate. Using laser confocal microscopy it is possible to corroborate the binding of the antibodies to the yeast and hyphal segments of the *Candida* cells.

30

Example 9: Antibody binding to *C. auris*

FIG.14 shows a microscopy image of antibodies bound to *C. auris* VPCI479/P/13 cells. Cells were observed with differential interference contrast microscopy (DIC) and fluorescent microscopy (secondary antibody Alexa 488). Sera was mixed with *C. auris* VPCI479/P/13 (South Asian clade) and coloured using Concavalin A – Alexa fluor 488. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻

35

³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval.

Conclusion:

As shown in FIG. 14, antibodies against modified Als3-NT protein-glucan bioconjugate are able to bind *C. auris* cells coated on plate and in Fig 13. Using laser confocal microscopy it is possible to corroborate the binding of the antibodies to *C. auris* cells.

Example 10: Inhibition of biofilm formation on 96-well plates

FIG. 15 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to inhibit biofilm formation of *C. albicans* hyphae on 96-well plates. Sera was added with *C. albicans* hyphae (SC5314) to plastic wells and incubated for 24hs at 37°C. After wash, viable cells were measured using XTT. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, all groups tested with AS03. NZW rabbits immunized three times on a two-week interval. Graph depicts Mean+SD. *: p<0.05, one-way ANOVA.

Conclusion:

As shown in FIG. 15, sera against modified Als3-NT protein-glucan bioconjugate inhibits biofilm formation of *C. albicans* to plastic. Significant reduction of biofilm formation was shown vs pre or post-III control sera.

Example 11: Neutrophile killing assay

FIG. 16 shows the capacity of antibodies against modified Als3-NT protein-glucan bioconjugate to mediate neutrophile killing of *C. albicans* hyphae. *C. albicans* hyphae (SC5314) were mixed with modified Als3-NT protein-glucan bioconjugate sera, added to neutrophiles and incubated. Then, neutrophiles were lysed and Candida CFU were counted after incubation in YPD agar. Modified Als3-NT protein-glucan bioconjugate: Als3¹⁸⁻³¹⁶-3S-Fba-bgluc^{d+}; Control indicates buffer immunized animals, pre sera of all animals was pooled to create a pre-immunization control. All groups tested with AS03. NZW rabbits immunized three times on a two-week interval. Graph depicts Mean+SD. ***: p<0.001; *: p<0.05, one-way ANOVA.

Conclusion:

As shown in FIG. 16, sera against modified Als3-NT protein-glucan bioconjugate mediates neutrophile killing of *C. albicans* hyphae. Significant increase in killing (reduction of CFUs) was shown vs pre or post-III control sera.

SEQUENCE LISTINGS

SEQ ID NO: 1 Full-length Wild type Als3 sequence from *Candida albicans* (with wild type Leader sequence)

MLQQYTLLLIYLSVATAKTITGVFNSFNSLTWSNAATYNYKGPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCV
FKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNLTTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK
CFTAGTNTVTFNDGGKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDV
QIDCSNIHVGITKGLNDWNYPVSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCA
5 GGYWQRAPFTLRWTGYRNSDAGSNGIVIVATTRTVTDSTTAVTTL PFDPNRDKTKTIEILKPIPTTTITTSYVGV
TTSYLTKTAPIGETATVIVIDIPYHTTTT VTSKWGTITSTTHTNPTDSIDTVIVQVPLPNPTVTTTEYWSQSFAT
TTTTITGPPGNTDVLIREPPNHTVTTTEYWSESYTTTSTFTAPPGGTD SVIIEPPNPTVTTTEYWSESYTTTTTV
TAPPGGTDTVIIEPPNHTVTTTEYWSQSYTTTTT VIAPPGGTD SVIIEPPNPTVTTTEYWSQSYATTTTITAPP
GETDVLIREPPNHTVTTTEYWSQSYATTTTITAPPGETDVLIREPPNHTVTTTEYWSQSYTTTTT VIAPPGGT
10 DSVIIEPPNPTVTTTEYWSQSYATTTTITAPPGETDVLIREPPNHTVTTTEYWSQSYATTTTITAPPGETDVL
IREPPNHTVTTTEYWSQSFATTTTITAPPGGTDTVIIEPPNHTVTTTEYWSQSFATTTTIIAPPGETDVLIREP
PNPTVTTTEYWSQSYTTATTVTAPPGGTDTVIYDMSSEISSFSRPHYTNHTTLWSTTWVIETKITETSC
DKGCSWVSSTRIVTIPNNIETPMVTNTVDTTTTTESTLQSPSGIFSESGVSVETESSTFTTAQTNPSVPTTESEV
FTTKGNNGNGPYESPSTNVKSSMDENSEFTTSTAASTSDIENETIATTGSVEASSPIISSSADETTT VTTTAE
15 SVIEQQTNNGGGNAPSATSTSSPSTTTTANSDSVITSTTSTNQSQSQSNSDTQQTTLSSQMTSSLVSLHMLT
TFDGSQSVIQHSTWLCGLITLLSLFI

SEQ ID NO: 2 Consensus sequence (artificial sequence)
G-S-G-G-G-D/E-X-N-Z-S/T-G-S-G-G

20

SEQ ID NO: 3 Fba sequence
YGKDVKDLFDYAQE

SEQ ID NO:4 Consensus sequence (artificial sequence)
25 K-D/E-X-N-Z-S/T
wherein X is Q (glutamine) and Z is A (alanine)

SEQ ID NO: 5 Consensus sequence (artificial sequence)
K-D-Q-N-A-T

30

SEQ ID NO: 6 Consensus sequence (artificial sequence)
K-D-Q-N-A-S

SEQ ID NO: 7 Consensus sequence (artificial sequence)
35 D-Q-N-A-T

SEQ ID NO: 8 Consensus sequence (artificial sequence)

J-U-B-D/E-X-N-Z-S/T-J-U-B

wherein X is Q (glutamine), Z is A (alanine), each of J and B comprises 1 to 5 Glycine (G) residues and U comprises 1 to 5 serine (S) residues

5

SEQ ID NO: 9 Consensus sequence (artificial sequence)

GSGGGDQ⁸NATGSGGG

10 SEQ ID NO: 10 Modified Als3-NT sequence (comprising amino acid 18-316 of SEQ ID NO: 1, with inserted glycosites underlined and fba sequence double-underlined) (artificial sequence)

SKTITGVFNSFNSLTWKDQNATYNYKGPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTA
HG VKYATCQFQAGEEKDQNA⁸SLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSCFTAGTNTVTFN
DGGKKISINVDFERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYSGTMGFANTYGDVQIDCSNIHVGITK
GLNDWNPVSSSEFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLR
15 WTGYRYGKDVKDLFDYAQEGSGGGDQ⁸NATGSGGG

SEQ ID NO: 11 Modified Als3-NT sequence (comprising amino acid 18-329 of SEQ ID NO: 1, with inserted glycosites underlined and fba sequence double-underlined) (artificial sequence)

20 SKTITGVFNSFNSLTWKDQNATYNYKGPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTA
HG VKYATCQFQAGEEKDQNA⁸SLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSCFTAGTNTVTFN
DGGKKISINVDFERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYSGTMGFANTYGDVQIDCSNIHVGITK
GLNDWNPVSSSEFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLR
WTGYRNSDAGSNGIVAYGKDVKDLFDYAQEGSGGGDQ⁸NATGSGGG

25 SEQ ID NO: 12 *Agrobacterium* sp. ZX09 SleC Sequence

MIHILYLAHDLSDPAIRRRVLTLLAGGARVTLAGFRRGQNR⁸LAEIEGVVPVVLGETADGQFLQRMAAVAKASLSL
GKVLNGIPAPDVVLARNLEMLALAKRAMSIYSGRPALVYECLDIHRLLLHEGKPGQMLNAAQRYFARDAKLLVTS
SPAFVEHYFKPVSGLDLPVLLQENKVLALDDTIAATPRPRAPAPGEPWKIGWFGALRCRKSLEILAEFARRMEGR
VEIILRGRPAYSEFADFDGFVAAAPHVHFHGPYKNPEDLA⁸AIYNEVQFTWAIDFFEEGQNSSWLLPNRLYEGCLY
30 GTLPIALAGTETARFIEKRNIGFVLQQAGADDLAALFNRMTPQTYADAFHTLSATDRKQWLTD⁸RDDCRLLVQQL
SSLAKSASGHAREAQFSPV

SEQ ID NO: 13 *Agrobacterium* sp. ZX09 SleE Sequence

35 MTDNTVTGTQYLKTVDIGICTYRRPALVATLLSLFELDVPEGVKVRLIVADNDEEPSAKASVDRLRETAPFEITYV
HCPKSNISIARNACLSECKADYLAFIDDD⁸ETAPPHWLAALLEKADETGAETVLGPVTAVYRDNAPGWMKRGDFH
STVPVWVNGEITGYTCNTLLRMEAPSVKGRRFALALGQSGGEDTHFFSHLHAAGGRIVFAEDAVLSEVPENRA

SFLWLAKRRFRSGQTHGRVLAEEKPGARRVQVWKAGSKVLYCALFAALSGFNAVRRNRYALRGALHMGSMMSG
AFGVREIRQYGAVEAT

SEQ ID NO: 14 *Agrobacterium* sp. ZX09 SleF Sequence

5 MENLTSPPDISFVIAAYNAADTIEAAVQSALDQQGVTLVIVDDRSADDTIPFVEAIAAIDPRVRLALEENRGP
GGARNAGIEAATGRWIAVLDSDDVIRPERSACMMCRAEAANADIAVDNLDVWYTDGRPMETMFPEEFLEERPVL
TLEDFISSNIFRSTFNFGYMKPMFRDFLNNEALRFREDIRIGEDYILLASALAAGGLCVIEPKPGYIYNIREGSIS
RVLELHHVEAMMRADDEFLSHYTLLPAAMDAQARARSLRLAHNFLTLENIKRRSVLGALKTTIRDPAVLGHLR
MPIAVRLRRLRDVAFAPAANTGVKRQIS

10

SEQ ID NO: 15 *Agrobacterium* sp. ZX09 SleU Sequence

MVSGSQPICVIAAKNASDTIDIAIRSALAEPEVGEVVIDDGSTDTTSDVAHAADDGTGRLRVVRFVNRGPSA
ARNHAISSAPLISILDADDDFFHGRFAAMLADDDWDLVADNIAFIQQSVPGASSMQPARFEPQARFLSLTEFVE
GNISRPGVERGETGFLKPVRRAFLDKHALRYDEALRLGEDYELYVRLAAGARYKVIRHCGYGAVIRGNSLSGR
15 HRTEDLRLLYEADRILAGCRLSAEETAILREHEKHIRAKFELRHFLDTKKQKGVSGALSHALARLPALPAITRGIW
SDKTARFRKAAPVRDVRYLLDGTGPS

SEQ ID NO: 16 *Agrobacterium* sp. ZX09 SleW Sequence

LINARGETMARFTVPIPYQKHGVLGRALASVFAQTYQDFDLVIVDDSPYPIDQELAELSQEQKDRILVIKQA
20 NAGPGGARNTGLDNVPGTDYVAFLDSDDIWTPDHLRNAAFALTTYGGECYWASMQASDEFYHFAISELEKN
EGAARLSEKPLVIELPDLASVMLRNWSFLHLSCMVIGRPLFEKIRFDPALRLAEDVLFCDASILASKRTLCCDDAG
AMRGMGVNIFHSIDNTSPEFLRQQFNTWVALDTLEGRFSRRPADVASIASYKNTARKQALWSQAGNLKRRKAP
EFGLLLKWAMRDPALLRAAFELGAGKIVRSR

25 SEQ ID NO: 17 *E. coli* 056 WfaP sequence

MELVSIIAAYNCKDTIYATVESALSQTYKNIEIIICDDSSSTDDTWDIINKIKDSRIICIKNNYCKGAAGARNCALKI
AKGRYIAFLDSDDYWVTTKISNQIHFMETEKVFFSYSNYYIEKDFVITGVFSSPPEINYGAMLYKCNIACTVILDR
TGVKNISFPYIDKEDYALWNLILSKGIKARNTNLVDTYRVRHAGSVSANKFKELIRQSNVLKSIKAKHHRICLFY
YAINGLIKHCFSYRDKRNA

30

SEQ ID NO: 18 *Klebsiella pneumoniae* Wzm sequence

VISAMPKGTRRTSMKYNLGYLFDLLVVITNKDLKVRYSMLGYLWSVANPLLFIYFIFKLVMRVQIPNYTVF
LITGLFPWQWFASSATNSLFSFIANAQIIKTVFPRSVIPLSNVMMEGHFLCTIPVIVVFLFVYGMTPSLSWVWG
IPLIAIGQVIFTFGVSIIFSTLNLFFRDLERFVSLGIMLMFYCTPILYASDMIPEKFSWIITYNPLASMILSWRDLFMN
35 GTLNYEYISILYFTGIILTVVGLSIFNKLKYRFAEIL

SEQ ID NO: 19 *Klebsiella pneumoniae* Wzt sequence

MHPVINFSHVTKEYPLYHHIGSGIKDLIFHPKRAFQLLKGRKYLAIEDVSFTVGKGEAVALIGRNGAGKSTSLGLV
AGVIKPTKGTVTTEGRVASMLELGGGFHPELTGRENIYLNATLLGLRRKEVQQRMERIIEFSELGEFIDEPIRVYSS
GMLAKLGFVSISQVEPDILIIDEVLAVGDI AFQAKCIQTIRDFKKRGVTILFVSHNMSDVEKICDRVIWIENHRLRE
5 VGSAERIIELYQAMA

SEQ ID NO: 20 *Campylobacter* PglB sequence

MLKKEYLKNPYLVLFAMII LAYVFSVFCRFYWWWASEFNEYFFNNQLMIISNDGYAFAEGARDMIAGFHQPND
LSYYGSSLSALTYWLYKITPFSFESII LYMSTFLSSLVVIPTILLANEYKRPLMGFVAALLASIANSYNNRTMSGYYD
10 TDMLVIVLPMFILFFMVRMILKKDFFSLIALPLFIGIYLWWYPSSYTLNVALIGLFLIYTLIFHRKEKIFYIAVILSSLT
LSNIAWFYQSAIIVLFALFALEQKRLNFMIIIGILGSATLIFLILSGGVDPIYLQKFYIFRSDSANLTQGFMYFNVN
QTIQEVENVDLSEFMRRISGSEIVFLFSLFGFVWLLRKHKSMIMALPILVLGFLALKGGLRFTIYSVPVMALGFGFL
LSEFKAIMVKYSQSLTSNVICVFATILTLAPVFIHIYNYKAPT VFSQNEASLLNQLKNIANREDYVVTWWDYGYPV
RYYSDVKTLVDGGKHLGKDNFFPSFALSKDEQAAANMARLSVEYTEKSFYAPQNDILKTDILQAMMKDYNQSNV
15 DLFLASLSKPDFKIDTPKTRDIYLYMPARMSLIFSTVASFSFINLDTGVLDKPFSTAYPLDVKNGEIYLSNGVLS
DDFRSKIGDNVSVNSIVEINSIKQGEYKITPIDDKAQFYIFYLKDSAIPYAQFILMDKTMFNSAYVQMFFLGNY
DKNLFDLVINSRDAKVFCLKI

SEQ ID NO: 21 *E. coli* flagellin (FlgI) signal sequence

20 MIKFLSALILLLVTTAAQA

SEQ ID NO: 22 *E. coli* outer membrane porin A (OmpA) signal sequence

MKKTAAIAVALAGFATVAQA

25 SEQ ID NO: 23 *E. coli* maltose binding protein (MalE) signal sequence

MKIKTGARILALSALTMMFSASALA

SEQ ID NO: 24 *E. coli* outer membrane porin A (OmpC) signal sequence

MKVKVLSELLVPALLVAGAANA

30

SEQ ID NO: 25 Als3-NT (1-316) sequence from *Candida albicans* (with wild type leader sequence)

MLQQYTLLLIYLSVATAKTITGVNSFNSLTWSNAATYNYKGPPTWNAVLGWSLDGTSASPGDTFTLNMPCV
FKFTTSQTSVDLTAHGKATCQFQAGEEFMTFSTLTCTVSNLTTPSIKALGTVTLPALFNVGGTGSSVDLEDSK
CFTAGTNTVTFNDGGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTS GTMGFANTYGDV
35 QIDCSNIHVGITKGLNDWNPVSSSEFSYTKTCSNGIFITYKNVPAGYRPFV DAYISATDVNSYTLSYANEYTCA
GGYWQRPFTLRWTGYR

SEQ ID NO: 26 Als3-NT (1-329) sequence from *Candida albicans* (with wild type leader sequence)

MLQQYTLLLIYLSVATAKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCV
FKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK
5 CFTAGTNTVTFNDGGKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDV
QIDCSNIHVGITKGLNDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCA
GGYWQRAPFTLRWTGYRNSDAGSNGIVIVA

SEQ ID NO: 27 Als3-NT (18-316) sequence from *Candida albicans*

10 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK CFTAGTNTVTFNDGG
KKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWT
GYR

15

SEQ ID NO: 28 Als3-NT (18-329) sequence from *Candida albicans*

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK CFTAGTNTVTFNDGG
KKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
20 DWNYPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWT
GYRNSDAGSNGIVIVA

SEQ ID NO: 29 Nucleotide sequence of *Campylobacter* PglB (codon optimized)

ATGCTGAAGAAAGAATATCTGAAAAATCCGTATCTGGTGCTGTTTCGCAATGATTATCCTGGCGTATGTGTTT
25 AGCGTGTTCTGTCGTTTCTACTGGGTGTGGTGGGCAAGTGAATTTAACGAATATTTCTTTAAACAACAGCTG
ATGATCATCTCCAATGATGGCTATGCCTTCGAGAAAGTGCCCGTGACATGATTGCAGGCTTTTCATCAGCCG
AACGATCTGAGTTATTACGGTAGCTCTCTGTCCGCCCTGACCTATTGGCTGTACAAAATCACGCCGTTTAGTT
TCGAATCCATTATCCTGTACATGAGTACCTTCCTGAGTTCCTGGTGGTTATTCCGACGATCCTGCTGGCAAA
TGAATATAAACGTCGCTGATGGGCTTTGTGGCGGCCCTGCTGGCTAGTATTGCGAACTCCTATTACAATCG
30 CACCATGAGTGGTTATTACGATACGGACATGCTGGTCATTGTGCTGCCGATGTTTCATCCTGTTTTTCATGGT
GCGTATGATTCTGAAAAAGGATTTCTTTAGCCTGATCGCTCTGCCGCTGTTTATTGGCATCTATCTGTGGTG
GTACCCGTCATCGTATACCCTGAACGTTGCGCTGATTGGTCTGTTTCTGATTTACACGCTGATCTTCCATCGC
AAGGAAAAGATCTTTTATATCGCGGTTATCCTGAGCTCTCTGACCCTGAGCAACATTGCTTGGTTTTATCAGT
CTGCGATTATCGTCATCCTGTTTGCCTGTTTCGCACTGGAACAAAAACGCTCTGAATTTTCATGATTATCGGCAT
35 TCTGGGTAGTGCTACCCTGATCTTTCTGATTCTGTCCGGCGGTGTTGATCCGATTCTGTACCAGCTGAAATT
TTATATCTTCCGCTCAGATGAATCGGCGAACCTGACCCAAGGCTTCATGTACTTCAACGTTAACCAGACGATC

CAAGAAGTGGAAAATGTTGATCTGAGCGAATTTATGCGTCGCATTAGTGGCTCCGAAATCGTTTTCTGTTCT
TCACTGTTTTGGTTTTCGTCTGGCTGCTGCGTAAACACAAGTCGATGATTATGGCCCTGCCGATCCTGGTGCTG
GGTTTTCTGGCACTGAAAGGCGGTCTGCGCTTTACCATTTACAGCGTTCCGGTCATGGCCCTGGGCTTTGGT
TTCTGCTGTCTGAATTTAAGGCAATCATGGTTAAAAAGTACTCACAGCTGACCTCGAACGTCTGCATTGTGT
5 TCGCCACCATCCTGACGCTGGCACC GGTTTCATCCATATCTACAACACAAGGCTCCGACGGTGTAGCC
AGAACGAAGCGTCGCTGCTGAATCAACTGAAGAACATTGCCAATCGTGAAGATTATGTCGTGACCTGGTGGG
ACTATGGCTACCCGGTGCGCTATTACAGCGATGTTAAACGCTGGTCGACGGCGGTAAACACCTGGGCAAGG
ACAACTTTTTCCCGAGCTTTGCTCTGTCTAAAGATGAACAGGCAGCTGCGAATATGGCGCGCTGTGAGTCG
AATACACCGAAAAGTCGTTTTATGCCCGCAGAATGATATTCTGAAAACGGACATCCTGCAGGCAATGATGA
10 AGGATTATAACCAAAGCAATGTTGACCTGTTCTGGCGTCACTGTGAAACCGGATTTTAAGATTGACACCCC
GAAAACGCGTGATATCTATCTGTACATGCCGGCTCGCATGAGTCTGATTTTTAGCACCGTCGCGAGCTTTTC
TTTCATCAACCTGGATACGGGCGTGCTGGACAAACCGTTTACCTTCTCAACGGCTACCCGCTGGATGTGAA
GAACGGCGAAATTTATCTGTGAATGGTGTTCCTGAGCGATGACTTTCTTTTCAAATCGGCGATAA
CGTTGTGAGCGTGAACAGCATCGTTGAAATTAATAGCATCAAACAGGGTGAATACAAGATTACCCGATCGA
15 TGACAAAGCGCAATTTTATATTTTCTACCTGAAGGACTCCGCTATTCCGTATGCGCAGTTCATCCTGATGGAT
AAAACCATGTTTAACTCTGCCTACGTTCAAATGTTTTCTGGGTAACACGATAAGAACCTGTTTGACCTGG
TCATTAATTCTCGCGATGCAAAGGTGTTTAACTGAAGATCTAA

SEQ ID NO: 30 Nucleotide sequence of sleW from *Agrobacterium* sp. ZX09

20 TTGATCAACGCAAGGGGCGAAACAATGGCAAGATTTACTGTCGTCATTCCCTACTATCAAAAGCAGCACGGT
GTCCTGGGACGTGCACTCGCATCGGTTTTTGCAGACTTACCAGGACTTCGATCTTGTCATCGTCGATGAC
GAATCGCCATACCCGATCGATCAGGAACCTTGCAGGAACTTTGCAGGAACAGAAAGACCGGATTCCTGTCATT
AAGCAGGCCAATGCCGGCCCGGGCGGCGCCGCAACACCGGTCTGGACAATGTGCTGACGGCACCGACTA
CGTTGCCTTTCTTGATTCCGACGATATCTGGACGCCGATCATCTGCGGAATGCGGCCTTTGCGCTCACGAC
25 CTATGGCGGCGAGTGCTACTGGGCGTCCATGCAGGCAAGTGACGAATTTTATTATCATTTCCGCAATTTCCGA
GCTGGAGAAGAATGAGGGTGCGGCACGGCTTTTCGAGAAGCCGCTGGTGATCGAACTGCCGGATCTCGCAA
GCGTCATGCTGCGGAACCTGGAGCTTCTGCATCTCTCCTGCATGGTGATCGGTCGTCCTTTTTCGAGAAGA
TCCGTTTCGATCCGGCGCTCAGACTGGCGGCGGAAGACGTGCTGTTTTCTGCGATTCCATCCTTGATCGA
AGCGGACACTGCTCTGTGACGACGCCGGCGCGATGCGCGCATGGGCGTCAATATCTTCCACAGCATCGACA
30 ATACCTCGCCGAATTCCTGCGCCAGCAGTTCAATACCTGGGTGGCGCTCGATACGCTGGAGGGACGTTTTT
CTCGCCGGCCGGCGATGTGGCGTCCATTGCTTCTATAAAAAACCCGCGCGCAACAGGCTCTCTGGAGCC
AGGCTGGCAACCTGAAACGGCGCAAGGCTCCCGAATTCGTTTTGCTCCTAAAATGGGCAATGCGCGATCCGG
CACTGCTGCGCGCGCTTTTCAAACCTGGCGCCGGAAAAATCGTCCGCTCCAGATGA

35 SEQ ID NO: 31 Nucleotide sequence of sleU from *Agrobacterium* sp. ZX09

ATGGTGAGCGGTTACAGCCCATTTGCGTCATCATCGCTGCAAAGAATGCATCTGATACCATCGATATCGCC
ATTCGTTCCGCCCTTGCGGAGCCAGAGGTCGGTGAAGTCGGTGATCGACGATGGTTCCACCGATACAACG
AGCGACGTGGCGCATGCGGCGGATGACGGCACGGGCCGCTGCGGGTGGTGCGATTGACGTCAACCGCG
GGCCATCGGCTGCGCGCAATCATGCCATCTCGATTTCTCCGCTCCCCTCATCAGCATTCTCGATGCGGACG
5 ATTTTTTCTTCCACGGCCGTTTTCGCCCATGCTGGCCGATGACGACTGGGACCTCGTGGCCGATAATATCG
CCTTCATTAGCAATCCGTTCCAGGCGCGTCTCCATGCAGCCTGCCCGCTTCGAGCCCCAGGCGCGTTTT
TGTCGCTGACGGAATTCGTGGAAGCAATATTTCCAGGCCTGGTGTGGAGCGCGGGGAAACCGGCTTCCTG
AAACCGGTGATACGCCGCGCCTTTCTCGACAAACATGCGCTGCGTTATGACGAGGCTCTGCGACTTGCGGAG
GATTACGAACTTTACGTGCGCGCGCTGGCGGCCGGCGCGCTACAAGGTCATCCGTATTGCGGTTACGG
10 TGCGATCGTTGCGGCAACTCGCTGAGTGGCCGCCACCGAACCGAAGATCTCCGTCTTCTCTACGAAGCAGA
CAGGGCGATTCTCGCCGTTGCCGGCTCTCGGCAGAGGAAACGGCGATCCTGCGCGAGCACGAGAAACACA
TCCGCGCCAAATTCGAGCTTCGCCATTTTCTCGATACAAAAAACAGAAGGGTGTGAGCGGAGCGCTGAGCC
ACGCCCTTGCGCGTCTGCCCGCCTGCCCGCATTACACGCGGTATCTGGAGCGACAAGACCGCCCGCTTCC
GCAAAGCGGCGCCGGTGCGGGACGTGCGTTATCTGCTGGACGGCACGCCGTTTCCTAA

15

SEQ ID NO: 32 Nucleotide sequence of sleF from *Agrobacterium* sp. ZX09

ATGGAACCTCACCTCTCCGCCAGACATCAGCTTCGTTATCGCCGCTATAATGCCGCCGATACGATTGAG
GCCGCGTTCAAAGCGCGCTCGATCAGCAGGGCGTGACGCTGGAAGTAATCGTCGTCGACGACCGCTCCGC
AGACGATACCATTCCTTTCGTTGAAGCGATCGCCGCAATCGATCCGCGCGTGCGGCTGCTTGCCTCGAAGA
20 AAACCGCGGTCCAGGCGCGCCCGCAACGCCGGCATCGAGGCCGCGACGGGGCGTGATCGCCGTGCTCG
ACTCCGACGATGTATCCGCCCGGAGCGCTCCGCTGCATGATGTGCCGGGCGGAAGCCGCAACGCTGACA
TCGCTGTCGATAATCTCGATGTCGTCTACACCGATGGCCGGCCGATGGAGACGATGTTTCCGAAGAGTTTC
TGGAGGAACGGCCTGTTCTCACGCTGGAGGACTTCATTTCTCCAACATCCTGTTCCGCTCCACCTTTAACTT
CGGCTACATGAAACCGATGTTCCGGCGCGATTTCTTAATAACGAAGCACTGCGCTTCCGTGAGGATATCCG
25 CATCGGCGAAGACTATATTCTTCTCGCTCGGCGCTTGCCGCTGGCGGCCTCTGCGTCATCGAACCGAAGCC
GGGTTACATCTACAAATATCCGCGAAGGTTGATTTCCCGTGCTCCTCGAATTCATCACGTCGAAGCGATGAT
GCGGGCGGACGAAGAATTCCTGAGCCACTACACCTTGCTGCCGGCCGCCATGGACGCGCAGCAGGCGAGAG
CCCGCAGCCTGCGGCTGGCGCATAATTTTTTGACTTTGGTGGAAAACATCAAGCGCCGCTCGGTACTCGGCG
CCTTGAAGACCACAATCCGAGATCCTGCCGTGCTGGGGCACCTGAGAATGCCGATTGCAGTGCGGCTGAGG
30 AGGCTGCGGGACGCCGTGTTTGCGCCCGCGGCGAATACAGGGGTGAAAAGGCAGATTTTCATAA

SEQ ID NO: 33 Nucleotide sequence of sleE from *Agrobacterium* sp. ZX09

ATGACCGACAACACCGTCACCGGCACGCAATATCTCAAGACCGTCGATATCGGCATCTGCACCTACAGACGC
CCGGCGCTTGTGCCCACTTCTGTCACTCTTCAGCTGGATGTGCCTGAAGGCGTGAAAGTTAGGCTGATT
35 GTGCCGATAATGACGAGGAGCCAGCGCAAAGGCAAGCGTCGATCGCCTGCGCGAAACCGCCCCCTTCGA
GATCACCTATGTGATTGCCGAAATCTAATATTTTCGATTGCCCGCAATGCCTGCCTGTCGGAATGCAAGGC

GGACTATCTCGCCTTTATCGATGACGACGAAACGGCGCCGCCACACTGGCTGGCCGCGCTTCTGGAAAAGGC
AGACGAGACCGGTGCAGAAACAGTTCTCGGCCCGTCACTGCGGTTTACCGGGACAACGCGCCGGGCTGGA
TGAAACGCGGAGATTTCCACTCGACCGTTCCGGTCTGGGTCAATGGCGAGATCATCACCGTTATACATGCA
ACACGCTGCTCAGGATGGAAGCGCCATCGGTAAAGGGCCGGCGCTTCGCGCTGGCGCTTGGCCAGAGCGGC
5 GGCGAAGACACACATTTCTTCTCGCATCTCCACGCCCGGGCGGCGGTATCGTCTTTCGCGAAGACGCTGTC
TTGTGCGAGCCGGTTCCGGAATAACGGGCAAGCTTTCTATGGCTTGCCAAGCGCCGGTTCCGTTCCGGCCAG
ACCCATGGACGGGTGCTGGCTGAAAAGAAACCCGGTGCGCGCCGCTCGTCCAGGTGGTGAAGCTGGTTC
GAAAGTGCTTTATTGCGCTCTTTTTGCCGCTTGAGCGGTTTCAATGCCGTACGCCGAACCGTTATGCGTT
GCGAGGCGCGCTGCATATGGGTTGATGAGCGGCGCTTCGGTGTGCGCGAAATCCGGCAATATGGTGCCG
10 TGGAGGCGACCTGA

SEQ ID NO: 34 Nucleotide sequence of sleC from *Agrobacterium* sp. ZX09
ATGATCCATATTCTCTACCTCGCGCATGATCTGTGACACCCCGCCATTGTCGGCGGGTGTGACGCTGCTT
GCAGGCGGGGCGCGGGTACGCTGGCCGGTTTCCGGCGGGACAGAACCGGTGGCGGAGATCGAAGGCG
15 TCGTACCTGTCTGTGCTCGGGGAAACCGCCGACGGGCAATTTCTGCAGCGCATGGCGGCAGTGGCGAAAGCC
AGCCTTTTCGCTCGGCAAGTCTTGAACGGAATTCGGGCACCCGACGTCGTTCTGCCAGGAACCTCGAAATG
CTGGCTCTGGCAAAGCGCGCCATGTCGATCTATTCCGGCCGCCCGGCGCTGGTTTACGAATGTCTCGATATT
CATGCGCTGCTGCTCCAGGAAGCAAGCCCGACAGATGCTGAATGCCGCGCAGCGTTATTTTCGACGCGAC
GCAAAGCTTCTGGTGACAAGTTCCTCCGGCATTCGTGGAGCATTATTTCAAGCCCGTGTGCGGCCCTCGACCTT
20 CCTGTCTGTTGCAGGAAAACAAGGTGCTGGCGCTCGACGACACCATTCGCCGACGCCAAGACCACGGGCG
CCCGCGCCCGGCGAACCATGGAATAACGGCTGGTTCGGCGCGCTTCGCTGCCGCAAATCGCTTGAAATTCTC
GCTGAGTTTCGCTCGCCGATGGAAGGCAGGGTCGAAATCATCCTGCGTGCCGCGCCGGCCTATTCGGAGTT
TGCTGATTTTCGATGGCTTCGTGGCGGCTGCTCCACATGTGCAATTTCCACGGACCTTACAAAAACCCCGAGGA
CCTAGCCGCCATCTATAACGAGGTGCAATTTACCTGGGCGATCGACTTTTTTCGAGGAAGGCCAGAATTCAG
25 CTGGCTGCTGCCAACCGGCTTTACGAGGGCTGCCTTTACGGCACTCTGCCGATTGCACTTGCTGGCACGGA
AACGGCTCGCTTCATTGAAAAACGCAATATCGGCTTCGTCTGCAACAGGCGGGCGCTGACGATCTTGCCGC
CCTCTTCAACCGGATGACCCCGCAAACCTATGCGGATGCCTTCCACACCCTGTCGGCAACAGACAGGAAACA
ATGGCTGACCGACCGCGACGATTGCCGTCTGCTGGTGAGCAATTGTCCTCTCTCGCCAAATCCGCTTCGG
CCACGCCCCTGAAGCGCAGTTTTACCCGTGTAG
30

SEQ ID NO: 35 Nucleotide sequence of wfaP from *E. coli* 056
ATGGAAGTGGTCTCTATCATCATTGCCGCTACAACCTGTAAGGACACGATCTATGCCACCGTCGAATCCGCCC
TGAGCCAAACCTACAAGAACATCGAAATTATCATTTGCGATGACAGCTCTACCGATGACACGTGGGATATTAT
TAACAAAATCAAGGACAGCCGTATCATTTGCATCAAGAACAACCTACTGTAAGGGTGCGGCGGGCGCGCTAA
35 CTGTGCTCTGAAATCGCGAAGGGCCGCTATATTGCCTTTCTGGATTGAGTACTGAGGTGACCACGAA
AATCTCGAACCAGATTCAATTCATGGAACCGAAAAGGTGTTTTTCAGCTACTCGAACTACTACATCGAAAAG

GATTTTCGTGATTACCGGCGTTTTTCAGTTCCTCCCGCCGGAAATCAACTATGGTGCTATGCTGAAATACTGCAATA
TCGCGTGTAGCACC GTTATTCTGGACCGCACGGGTGTCAAGAACATCTCTTTTCCGTACATTGATAAGGAAG
ACTACGCCCTGTGGCTGAATATCCTGTCAAAAGGCATTAAGGCACGTAACACCAATCTGGTCGATACCTATTA
CCGCGTCCATGCCGGTAGCGTGTCTGCAAACAAATTCAAGGAACTGATTCGTCAAAGTAATGTTCTGAAATC
5 CATCGGCATTAAGGCTCATCCGCATCATTTGCCTGTTTTATTACGCGATCAACGGTCTGATTAAACACTGT
TTCTCCTATCGTGATAAGCGCAATGCCTAA

SEQ ID NO: 36 Nucleotide sequence of wzm from *Klebsiella pneumoniae*
ATGAAGTACAATTTAGGGTATTTATTTGATTTACTTGTTGTCATAACAAATAAAGATCTAAAAGTGCGCTATA
10 AGAGCAGCATGCTAGGCTATTTATGGTCAGTAGCAAATCCATTGCTTTTTGCCATGATTTATTATTTTATATT
TAAGCTGGTAATGAGAGTACAAATTCAAATTATACCGTTTTCTCATTACCGGCTTGTTCCGTGGCAATGG
TTTGCCAGTTCGGCCACTAACTCATTATTTTCATTATCGCTAACGCTCAAATTATCAAGAAGACAGTTTTTC
CCCGTCCGTGATTCGCTAAGTAATGTAATGATGGAAGGGTTGCATTTTCTTTGTACCATCCCGTTATTG
TTGTCTTTCTTTTGTATGGCATGACGCCGTCTTGTCTGGGTTTGGGGTATACCTCTCATTGCTATTGG
15 CCAGGTGATTTTACCTTTGGTGTTCATCATCTTTTCAACGCTGAACCTGTTTTTCCGTGACCTGGAGCGC
TTTGTCAGTCTGGGGATTATGCTGATGTTTTATTGTACGCCGATTTTATATGCGTCTGATATGATTCCGGAAA
AATTTAGCTGGATAATTACCTACAATCCGCTAGCGAGTATGATTCTTAGTTGGCGTGATTTATTATGAATGG
GACTCTTAATTATGAGTATATTTCTATACTCTATTTACGGGAATCATTTTGACGGTTGTGGTTTGTCTATT
TTCAATAAATTAATAATATCGATTTGCAGAGATCTTGTA

20
SEQ ID NO: 37 Nucleotide sequence of wzt from *Klebsiella pneumoniae*
ATGCACCCAGTTATTAACCTCAGTCATGTTACAAAAGAGTATCCTCTGTACCATCATATTGGCTCAGGAATCA
AAGATTTAATTTTCCATCCGAAACGCGCTTTTCAATTGCTGAAGGGGCGGAAATATTTAGCTATCGAAGACGT
ATCCTTTACAGTTGGCAAAGGTGAGGCTGTTGCTCTGATTGGACGTAATGGGGCAGGAAAGAGTACCTCTCT
25 TGGCCTGGTTGCCGGCGTGATTAAGCCAATAAGGGAACCGTCACCACTGAAGGACGGGTGGCATCGATGC
TTGAACTCGGCGGAGGCTTTCATCCGGAACCTACCGGGCGTGAGAATATTTACCTGAATGCTACTCTGCTGG
GCCTTCGGCGTAAAGAGGTCCAGCAACGTATGGAACGTATTATTGAATTTTCCGGAACGGGAGAAATTCATAG
ACGAGCCAATCAGAGTGTACTCAAGCGGAATGCTAGCTAAGTTAGGTTTTTCGGTCATCAGTCAGGTTGAAC
CGGATATTTTAATTATTGATGAAGTTCTGGCAGTAGGTGATATCGCTTTTCAGGCAAAATGATTTCAGACCAT
30 AAGAGATTTTAAGAAAAGAGGCGTGACAATATTGTTTGTAGCCACAATATGAGTGACGTTGAAAAAATCTG
CGACAGAGTCATCTGGATCGAAAATCATAGGCTCAGAGAAGTGGGGTCTGCAGAGCGAATCATTTGAACGTGA
CAAGCAAGCAATGGCTTAA

SEQ ID NO: 38 Als3-NT (1-316) sequence from *Candida albicans* (with wild type leader and Fba sequence)

MLQQYTLLLIYLSVATAKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCV
FKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK
5 CFTAGTNTVTFNDGGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDV
QIDCSNIHVGITKGLNDWNPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCA
GGYWQRAPFTLRWTGYRYGKDVKDLFDYAOE

10 SEQ ID NO: 39 Als3-NT (1-329) sequence from *Candida albicans* (with wild type leader and Fba sequence)

MLQQYTLLLIYLSVATAKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCV
FKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSK
CFTAGTNTVTFNDGGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDV
QIDCSNIHVGITKGLNDWNPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCA
15 GGYWQRAPFTLRWTGYRNSDAGSNGIVIVAYGKDVKDLFDYAOE

SEQ ID NO: 40 Als3-NT (18-316) sequence from *Candida albicans* (with Fba sequence)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
20 KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAAGGYWQRAPFTLRWT
GYRYGKDVKDLFDYAOE

SEQ ID NO: 41 Als3-NT (18-329) sequence from *Candida albicans* (with Fba sequence)

25 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAAGGYWQRAPFTLRWT
GYRNSDAGSNGIVIVAYGKDVKDLFDYAOE

30

SEQ ID NO: 42 Full-length Wild type Als3 sequence from *Candida albicans* (without Leader sequence)

KTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
VKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGGK
KISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
35 WNPVSSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAAGGYWQRAPFTLRWTG
YRNSDAGSNGIVATTRVTVDSTTAVTTLPFDPNRDKTKTIEILKPIPTTTITTSYGVTTSYLTKTAPIGETATVI

VDIPYHTTTT VTSKWTGTITSTTHTNPTDSIDTVIVQVPLNPVT TTEYWSQSFATTTTITGPPGNTDTVLIR
EPPNHTVT TTEYWSESYTTTSTFTAPPGGTD SVIIKEPPNPTVT TTEYWSESYTTT TTVTAPPGGTD TVIIREPPN
HTVT TTEYWSQSYTTT TTVIAPPGGTD SVIIREPPNPTVT TTEYWSQSYATTTTITAPPGETDTVLIREPPNHTVT
TTEYWSQSYATTTTITAPPGETDTVLIREPPNHTVT TTEYWSQSYTTT TTVIAPPGGTD SVIIKEPPNPTVT TTEY
5 WSQSYATTTTITAPPGETDTVLIREPPNHTVT TTEYWSQSYATTTTITAPPGETDTVLIREPPNHTVT TTEYWSQ
SFATTTT TTVTAPPGGTD TVIIREPPNHTVT TTEYWSQSFATTTTITAPPGETDTVLIREPPNPTVT TTEYWSQSYTT
ATTVTAPPGGTD TVIIYDTMSSSEISSFSRPHYTNHTTLWSTTWVIETKITETSCGDKGCSWVSVSTRIVTIP
NNIETPMVTNTVD TTTTTESTLQSPSGIFSESGVSVETESSTFTTAQTNPSPVPTTESEVFTTKGNNGNGPYESPS
TNVKSSMDENSEFTTSTAASTSTD IENETIATTGSVEASSPIISSADETTT TTTTAESTSVIEQQTNNNGGNGAP
10 SATSTSSPSTTTTANSDSVITSTTSTNQSQSQSNSDTQQTTLSQQMTSSLVSLHMLTTFDGSGSVIQHSTWLCCG
LITLLSLFI

SEQ ID NO: 43 (Als3_18-316-N)

SGGGGGDQ NATGGGGKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMP
15 CVFKFTTSQTSVDLTAHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLED
SKCFTAGTNTVT FNDGGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYG
DVQIDCSNIHVGITKGLNDWNYPVSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYT
CAGGYWQRAPFTLRWTGYRHHHHHHHHHH

20 SEQ ID NO: 44 (Als3_18-316-C)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMP CVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLED SKCFTAGTNTVT FNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWT
25 GYRGGGGDQ NATGGGGHHHHHHHHHH

SEQ ID NO: 45 (Als3_18-316-Mut1)

SKTITGVFNSFNSLTWDQ NATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMP CVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLED SKCFTAGTNTVT FNDGG
30 KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSESFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWT
GYRHHHHHHHHHH

SEQ ID NO: 46 (Als3_18-316-Mut2)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTDQNATSVDLT
AHGVKYATCQFQAGEEFMTFSTLTCTVSNTLTSPKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFND
GGKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKG
LNDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLR
5 WTGYRHHHHHHHHHH

SEQ ID NO: 47 (Als3_18-316-Mut3)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAD
QNATGVKYATCQFQAGEEFMTFSTLTCTVSNTLTSPKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFN
10 NDGGKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGIT
KGLNDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLR
RWTGYRHHHHHHHHHH

SEQ ID NO: 48 (Als3_18-316-Mut4)

15 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEDQNASTLTCTVSNTLTSPKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDG
GKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGL
NDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLRW
TGYRHHHHHHHHHH

20

SEQ ID NO: 49 (Als3_18-316-Mut5)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLDQNATIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFN
DGGKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITK
25 GLNDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLR
WTGYRHHHHHHHHHH

SEQ ID NO: 50 (Als3_18-316-Mut6)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
30 GVKYATCQFQAGEEFMTFSTLTCTVSNTLTSPIDANATVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDG
GKKISINVD FERSNVD PKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGL
NDWNPVSSSESFSYTKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLRW
TGYRHHHHHHHHHH

35 SEQ ID NO: 51 (Als3_18-316-Mut7)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGDQNVTFNDG
GKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTMGFANTYGDVQIDCSNIHVGITKGL
NDWNPVSSSESFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLRW
5 TGYRHHHHHHHHHHH

SEQ ID NO: 52 (Als3_18-316-Mut8)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDG
10 QNATGKKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTMGFANTYGDVQIDCSNIHVGI
TKGLNDWNPVSSSESFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFT
LRWTGYRHHHHHHHHHHH

SEQ ID NO: 53 (Als3_18-316-Mut9)

15 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKIDINVTFERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTMGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSESFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLRWT
GYRHHHHHHHHHHH

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SEQ ID NO: 54 (Als3_18-316-Mut10)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FEDSNVTPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTMGFANTYGDVQIDCSNIHVGITKGLN
25 DWNYPVSSSESFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTLRWT
GYRHHHHHHHHHHH

SEQ ID NO: 55 (Als3_18-316-Mut11)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
30 GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCADQNATGYTSGMTMGFANTYGDVQIDCSNIHVGIT
KGLNDWNPVSSSESFSYTKCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTL
RWTGYRHHHHHHHHHHH

35 SEQ ID NO: 56 (Als3_18-316-Mut12)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTDQ NATGDVQIDCSNIHVGIT
KGLNDWNYPVSSSEFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTL
5 RWTGYRHHHHHHHHHHH

SEQ ID NO: 57 (Als3_18-316-Mut13)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
10 KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKDQN
ATGLNDWNYPVSSSEFSYTKTCSSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFT
LRWTGYRHHHHHHHHHHH

SEQ ID NO: 58 (Als3_18-316-Mut14)

15 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSEFSYDQ NATKTCSNGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFT
LRWTGYRHHHHHHHHHHH

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SEQ ID NO: 59 (Als3_18-316-Mut15)

SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
25 DWNYPVSSSEFSYTKC SSDQ NATGIFITYKNVPAGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFTL
RWTGYRHHHHHHHHHHH

SEQ ID NO: 60 (Als3_18-316-Mut16)

30 SKTITGVFNSFNSLTWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSSEFSYTKC SSNGIFITYKNVPDQ NATGYRPFVDAYISATDVNSYTLSYANEYTCAGGYWQRAPFT
LRWTGYRHHHHHHHHHHH

35 SEQ ID NO: 61 (Als3_18-316-Mut17)

SKTITGVFNSFNLSLTSWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSESFSYTKTCCSNGIFITYKNVPAGYRPFVDAYISATDVDQ NATY T LSYANEYTCAGGYWQRAPFTL
5 RWTGYRHHHHHHHHHHH

SEQ ID NO: 62 (Als3_18-316-Mut18)

SKTITGVFNSFNLSLTSWSNAATYNYKPGTPTWNAVLGWSLDGTSASPGDTFTLNMPCVFKFTTSQTSVDLTAH
GVKYATCQFQAGEEFMTFSTLTCTVSNTLTPSIKALGTVTLPLAFNVGGTGSSVDLEDSKCFTAGTNTVTFNDGG
10 KKISINVD FERSNVDPKGYLTDSRVIPSLNKVSTLFVAPQCANGYTSGMTGFANTYGDVQIDCSNIHVGITKGLN
DWNYPVSSESFSYTKTCCSNGIFITYKNVPAGYRPFVDAYISATDVNSY T LSYANEYTCADQ NATGGYWQRAPF
TLRWTGYRHHHHHHHHHHH

SEQ ID NO: 63 *Erwinia carotovora*s pectate lyase (PelB)

15 MKYLLPTAAAGLLLLAAQPAMA

SEQ ID NO: 64 Heat labile *E. coli* enterotoxin LTIIb

MSFKKIIKAFVIMAALVSVQAHA

20 SEQ ID NO: 65 *Bacillus subtilis* endoxylanase XynA

MFKFKKKFLVGLTAAAFMSISMFSATASA

SEQ ID NO: 66 *E. coli* DsbA

MKKIWLALAGLVLAFSASA

25

SEQ ID NO: 67 TolB

MKQALRVAFGFLILWASVLHA

SEQ ID NO: 68 SipA

30 MKMNKKVLLTSTMAASLLSVASVQAS

SEQ ID NO: 69 Nucleotide sequence of modified Als3-NT protein (comprising amino acid 18-316 of
SEQ ID NO: 1)

AGCAAGACCATCACCGCGTTTTCAACAGCTTCAACAGCCTGACCTGGAAAGATCAGAACGCGACCTACAAC
35 TACAAGGGCCCGGGCACCCGACCTGGAACGCGGTGCTGGGTGGAGCCTGGATGGTACCAGCGCGAGCCC

GGGTGACACCTTCACCCTGAACATGCCGTGCGTTTTCAAGTTTACCACCAGCCAGACCAGCGTGGATCTGAC
CGCGCACGGTGTTAAATACGCGACCTGCCAGTTTCAAGCGGGCGAGGAAAAAGATCAGAACGCGAGCACCT
GACCTGCACCGTGAGCAACACCCTGACCCCGAGCATCAAAGCGCTGGGTACCGTTACCCTGCCGCTGGCGTT
CAACGTGGGTGGCACCGGCAGCAGCGTTGACCTGGAGGATAGCAAATGCTTCACCGCGGGTACCAACACCG
5 TGACCTTTAACGACGGTGGCAAGAAAATCAGCATTAACTGGACTTTGAACGTAGCAACGTTGATCCGAAGG
GTTATCTGACCGACAGCCGTGTTATCCCGAGCCTGAACAAAGTGAGCACCTGTTGCTTGCGCCGAGTGCG
CGAACGGTTACACCAGCGGTACCATGGGCTTTGCGAACACCTATGGCGACGTGCAAATTGATTGCAGCAACA
TCCACGTTGGTATTACCAAGGGCCTGAACGATTGGAACCTACCCGGTGAGCAGCGAGAGCTTCAGCTATACCA
AGACCTGCAGCAGCAACGGTATCTTTATTACCTATAAAAACTGCCGGCGGGCTACCGTCCGTTGCTTGATG
10 CGTATATCAGCGCGACCGACGTTAACAGCTACACCCTGAGCTATGCGAACGAATACACCTGCGCGGGTGGCT
ACTGGCAACGTGCGCCGTTTACCCTGCGTTGGACCGGCTATCGTTATGGCAAAGATGTGAAAGATCTGTTTG
ATTATGCGCAGGAAGGCTCTGGCGGCGCGATCAGAACGCGACCGGCTCCGGCGGCGGC

SEQ ID NO: 70 Nucleotide sequence of modified Als3-NT protein (comprising amino acid 18-329 of
15 SEQ ID NO: 1)
AGCAAGACCATCACCGCGTTTTCAACAGCTTCAACAGCCTGACCTGGAAAGATCAGAACGCGACCTACAAC
TACAAGGGCCCGGGCACCCGACCTGGAACGCGGTGCTGGGTTGGAGCCTGGATGGTACCAGCGCGAGCCC
GGGTGACACCTTCACCCTGAACATGCCGTGCGTTTTCAAGTTTACCACCAGCCAGACCAGCGTGGATCTGAC
CGCGCACGGTGTTAAATACGCGACCTGCCAGTTTCAAGCGGGCGAGGAAAAAGATCAGAACGCGAGCACCT
20 GACCTGCACCGTGAGCAACACCCTGACCCCGAGCATCAAAGCGCTGGGTACCGTTACCCTGCCGCTGGCGTT
CAACGTGGGTGGCACCGGCAGCAGCGTTGACCTGGAGGATAGCAAATGCTTCACCGCGGGTACCAACACCG
TGACCTTTAACGACGGTGGCAAGAAAATCAGCATTAACTGGACTTTGAACGTAGCAACGTTGATCCGAAGG
GTTATCTGACCGACAGCCGTGTTATCCCGAGCCTGAACAAAGTGAGCACCTGTTGCTTGCGCCGAGTGCG
CGAACGGTTACACCAGCGGTACCATGGGCTTTGCGAACACCTATGGCGACGTGCAAATTGATTGCAGCAACA
25 TCCACGTTGGTATTACCAAGGGCCTGAACGATTGGAACCTACCCGGTGAGCAGCGAGAGCTTCAGCTATACCA
AGACCTGCAGCAGCAACGGTATCTTTATTACCTATAAAAACTGCCGGCGGGCTACCGTCCGTTGCTTGATG
CGTATATCAGCGCGACCGACGTTAACAGCTACACCCTGAGCTATGCGAACGAATACACCTGCGCGGGTGGCT
ACTGGCAACGTGCGCCGTTTACCCTGCGTTGGACCGGCTATCGTAACAGCGATGCGGGTAGCAACGGCATCG
TGATCGTTGCGTATGGCAAAGATGTGAAAGATCTGTTTGATTATGCGCAGGAAGGCTCTGGCGGCGGCGAT
30 CAGAACGCGACCGGCTCCGGCGGCGGC

Consensus sequence (artificial sequence)

K-D/E-X-N-Z-S/T

35 Consensus sequence (artificial sequence)

J-U-B-D/E-X-N-Z-S/T-J-U-B

CLAIMS

1. A modified Agglutinin-like sequence 3 (Als3) protein comprising amino acid residues 18-316 of SEQ ID NO: 1 or an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, modified in that the amino acid sequence comprises one or more consensus sequences comprising an amino acid sequence of D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.
5
2. The modified Als3 protein of claim 1, wherein the modified Als3 protein is of *Candida albicans* and wherein the modified Als3 protein further comprises at least one Fructose biphosphate aldolase-1 (Fba) peptide comprising an amino acid sequence of YGKDVKDLFDYAE (SEQ ID NO: 3) or an amino acid sequence at least 70%, 80%, 85%, 90%, or 92% identical to SEQ ID NO: 3.
10
3. The modified Als3 protein of any of claims 1 and 2, wherein X is Q (glutamine), Z is A (alanine) and the one or more consensus sequences are selected from the group consisting of KDQNAS (SEQ ID NO: 5), KDQNAS (SEQ ID NO: 6) and DQNAS (SEQ ID NO: 7), and wherein the modified Als3 protein comprises at least one additional consensus sequence comprising an amino acid sequence of J-U-B-D/E-X-N-Z-S/T-J-U-B, wherein X and Z are independently any amino acid except proline and J, U and B independently comprise 1 to 5 naturally occurring amino acid residues, wherein the at least one consensus sequence has been added next to C-terminal amino acid residue of SEQ ID NO: 3 or at an equivalent position within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 3.
15
20
4. A modified Als3 protein comprising an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11.
- 25 5. A conjugate comprising the modified Als3 protein of any of claims 1 to 4 and at least one saccharide antigen, optionally wherein the conjugate is a bioconjugate.
6. A modified Als3 protein of *Candida albicans* consisting of: (1) an amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 11; and (2) at least one saccharide antigen of *Candida*, wherein the at least one saccharide antigen is a β -1,3 glucan polymer consisting of at least six consecutive β -1,3 linked glucose molecules, and wherein the at least one saccharide antigen is linked to at least one of three asparagine residues at positions 20, 92, and 324 of SEQ ID NO: 10 or positions 20, 92, and 337 of SEQ ID NO: 11.
30

7. A polynucleotide sequence encoding the modified Als3 protein of any of claims 1 to 4.
8. A vector comprising the polynucleotide sequence of claim 7.
9. An immunogenic composition comprising the modified Als3 protein of any of claims 1 to 4 and 6, the conjugate of claim 5, or the bioconjugate of claim 5.
- 5 10. A *Candida albicans* vaccine comprising: (1) the modified Als3 protein of any of claims 1 to 4; (2) at least one *Candida albicans* saccharide antigen linked to said modified Als3 protein; and, optionally, (3) a pharmaceutically acceptable carrier or adjuvant.
- 10 11. A method for treatment or prevention of *Candida albicans* infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the modified Als3 protein of any of claim 1 to 4 and 6, the conjugate of claim 5, the bioconjugate of claim 5, the immunogenic composition of claim 9, or the vaccine of claim 10.
- 15 12. A method for immunizing a subject against *Candida albicans* infection, the method comprising administering to the subject an immunoprotective dose of the modified Als3 protein of any of claims 1 to 4 and 6, the conjugate of claim 5, the bioconjugate of claim 5, the immunogenic composition of claim 9, or the vaccine of claim 10.
- 20 13. A method for inducing immune response to *Candida albicans* infection in a subject, the method comprising administering to the subject a therapeutically or prophylactically effective amount of the modified Als3 protein of any of claims 1 to 4 and 6, the conjugate claim 5, the bioconjugate of claim 5, the immunogenic composition of claim 9, or the vaccine of claim 10.
- 20 14. The modified Als3 protein of any of claims 1 to 4 and 6, the conjugate of claim 5, the bioconjugate of claim 5, the immunogenic composition of claim 9, or the vaccine of claim 10 for use in treatment or prevention of a disease caused by *Candida albicans* infection.
- 25 15. A method for increasing expression level of the modified Als3 protein of any of claims 1 to 4 and 6, the method comprising substituting the one or more consensus sequences for the amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1, wherein the modified Als3 protein exhibits an increased expression level relative to a control

Als3 protein which does not comprise one or more consensus sequences substituted for amino acids between amino acid residues 104-108 of amino acid residues 18-316 of SEQ ID NO: 1 or at equivalent positions within an amino acid sequence at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to amino acid residues 18-316 of SEQ ID NO: 1.

- 5
16. A host cell comprising:
- 10

i.

ii.

iii.

iv.

a nucleotide sequence encoding one or more heterologous glycosyltransferase(s) capable of synthesizing a β -1,3 glucan polymer;

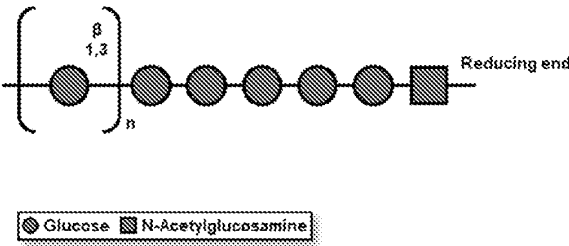
a nucleotide sequence encoding a glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule;

a nucleotide sequence encoding a heterologous oligosaccharyl transferase; and

optionally, a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are independently any amino acid except proline.

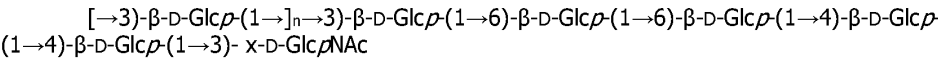
17. A saccharide which is a glucan having the structure:

15



wherein n is 2-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

- 20
18. A saccharide which is a glucan having the structure:



wherein n is 4-100, 4-50, 4-35, 4-25, 6-100, 6-50, 6-35, or 6-25.

- 25
19. A method of producing a β -1,3 glucan polymer in a prokaryotic host cell, the method comprising the steps of introducing and expressing in the host cell:

- 5 i. a nucleotide sequence encoding a first glycosyltransferase capable of covalently bonding a glucose molecule to an N-acetyl glucosamine (GlcNAc) molecule, wherein the first glycosyltransferase is WfaP from *E. coli* O56;
- ii. a nucleotide sequence encoding additional glycosyltransferases capable of synthesizing a fungal β -1, 3 glucan, wherein the additional glycosyltransferases comprise SleC, SleE, SleF, SleU and SleW from rhizobia, optionally from *Agrobacterium*, optionally from *Agrobacterium* sp. ZX09, and wherein the host cell produces more SleW than SleC, SleE, SleF or SleU; and
- 10 iii. optionally, a nucleotide sequence encoding a translocase capable of translocating the β -1, 3 glucan to periplasmic side of an inner membrane of the prokaryotic host cell, wherein the translocase comprises Wzm-Wzt from *Klebsiella* sp., optionally from *Klebsiella pneumoniae*,
- wherein the β -1,3 glucan polymer is linked to a lipid carrier via the GlcNAc and wherein the β -1,3 glucan polymer comprises at least four β -1,3 linked glucose molecules.
- 15 20. A method of producing a bioconjugate in a prokaryotic host cell, the method comprising the steps of:
- a. obtaining a prokaryotic host cell of claim 16 that produces a β -1,3 glucan polymer; and
- b. further introducing and expressing in the host cell:
- 20 i. a nucleotide sequence encoding a modified carrier protein comprising a glycosylation site comprising a consensus sequence D/E-X-N-Z-S/T, wherein X and Z are any amino acid except proline, and wherein the modified carrier protein further comprises an N-terminal bacterial signal sequence capable of transporting the modified carrier protein to the periplasmic side of the inner
- 25 membrane of the prokaryotic host cell; and
- ii. a nucleotide sequence encoding an oligosaccharyl transferase capable of producing a bioconjugate by transferring the β -1,3 glucan polymer from a lipid carrier to the modified carrier protein, wherein the oligosaccharyl transferase is PglB from *Campylobacter*, optionally from *Campylobacter jejuni* or
- 30 *Campylobacter coli*.