applications including antibody production, cytokine production, and for treatment of metabolic disorders such as lysosomal storage disorders.

Featured is~~In one aspect, this document features~~ a method of producing a fungal cell (e.g., *Yarrowia lipolytica* or *Arxula adeninivorans*) capable of producing proteins comprising $GlcNAcMan_5GlcNAc_2$ N-glycans. The method includes providing a fungal cell genetically engineered to produce proteins comprising $Man_5GlcNAc_2$ N-glycans; and introducing into the cell a nucleic acid encoding a GlcNAc-transferase I, wherein the nucleic acid includes a nucleotide targeting sequence to target the encoded GlcNAc-transferase I to an intracellular compartment (e.g., Golgi apparatus), wherein expression of the GlcNAc-transferase I in the fungal cell produces proteins including $GlcNAcMan_5GlcNAc_2$ N-glycans.

In one aspect, there is provided a method of producing a *Yarrowia lipolytica* cell for producing proteins comprising $GlcNAcMan_5GlcNAc_2$ N-glycans, said method comprising:

a) providing a *Yarrowia lipolytica* cell genetically engineered to produce proteins comprising $Man_5GlcNAc_2$ N-glycans;

b) introducing into said cell a nucleic acid encoding a GlcNAc-transferase I, wherein said nucleic acid comprises a nucleotide sequence encoding a targeting sequence to target the encoded GlcNAc-transferase I to an intracellular compartment, wherein expression of said GlcNAc-transferase I in said *Yarrowia lipolytica* cell produces proteins comprising $GlcNAcMan_5GlcNAc_2$ N-glycans.

The method further can include introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein modified to include the $GlcNAcMan_5GlcNAc_2$ N-glycans. The target protein can bind to an Fc receptor. The target protein can be an antibody or fragment thereof. The target protein can be a therapeutic glycoprotein. The target protein can be Interferon-β, GM-CSF, Interferon γ, or erythropoietin.

The fungal cell genetically engineered to produce proteins containing $Man_5GlcNAc_2$ N-glycans can be deficient in OCH1 activity and include a nucleic acid encoding an α-1,2-mannosidase, wherein the nucleic acid encoding the α-1,2-

mannosidase includes a nucleotide sequence encoding a targeting sequence to target the encoded α-1,2-mannosidase to the endoplasmic reticulum. The targeting sequence can be an HDEL sequence.

The method further can include introducing into a cell a nucleic acid encoding a mannosidase II, wherein the nucleic acid encoding the mannosidase II includes a nucleotide sequence encoding a targeting sequence to target the encoded mannosidase II to the Golgi apparatus, wherein expression of the mannosidase II in the fungal cell produces proteins containing $GlcNAcMan_3GlcNAc_2$ N-glycans.

The method further can include introducing into a cell a nucleic acid encoding a galactosyltransferase, wherein the nucleic acid encoding the galactosyltransferase includes a nucleotide sequence encoding a targeting sequence to target the encoded galactosyltransferase to the Golgi apparatus, wherein expression of the

galactosyltransferase in the fungal cell produces proteins containing $GalGlcNAcMan_5GlcNAc_2$ or $GalGlcNAcMan_3GlcNAc_2$ N-glycans. The galactosyltransferase can be a fusion of a UDP-Glc-4-epimerase and the catalytic domain of a β-1,4-galactosyltransferase I. Such a method further can include introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein modified to contain $GalGlcNAcMan_5GlcNAc_2$ or $GalGlcNAcMan_3GlcNAc_2$ N-glycans. The methods can include isolating the target protein modified to contain the $GalGlcNAcMan_5GlcNAc_2$ or $GalGlcNAcMan_3GlcNAc_2$ N-glycans.

Also featured is~~In another aspect, this document features~~ a method of producing a target protein containing $GlcNAcMan_3GlcNAc_2$ N-glycans. The method includes providing a fungal cell (e.g., *Yarrowia lipolytica* or *Arxula adeninivorans*) genetically engineered to include a nucleic acid encoding a GlcNAc-transferase I, an α-1,2-mannosidase, and a mannosidase II, wherein the nucleic acid includes a nucleotide sequence encoding a targeting sequence, or nucleotide sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment, wherein the fungal cell is deficient in OCH1 activity; and introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein containing $GlcNAcMan_3GlcNAc_2$ N-glycans.

In one aspect, there is provided a method of producing a target protein comprising $GlcNAcMan_3GlcNAc_2$ N-glycans, said method comprising:

a) providing a *Yarrowia lipolytica* cell genetically engineered to comprise a nucleic acid encoding a GlcNAc-transferase I, an α-1,2-mannosidase, and a mannosidase II, wherein said nucleic acid comprises nucleotide sequences encoding targeting sequences to target each encoded protein to an intracellular compartment, wherein said *Yarrowia lipolytica* cell is deficient in OCH1 activity; and

b) introducing into said cell a nucleic acid encoding a target protein, wherein said cell produces said target protein comprising said $GlcNAcMan_3GlcNAc_2$ N-glycans.

The nucleic acid encoding the α-1,2-mannosidase can include an endoplasmic reticulum targeting sequence to target the encoded α-1,2-mannosidase to the endoplasmic reticulum. For example, the targeting sequence can be an HDEL sequence. The nucleic acid encoding the GlcNAc-transferase I and the mannosidase II can include a Golgi

targeting sequence, or Golgi targeting sequences, to target the encoded GlcNAc-transferase I and mannosidase II to the Golgi apparatus. The target protein can bind to an Fc receptor. The target protein can be an antibody or fragment thereof. The target protein can be a therapeutic glycoprotein. The target protein can be Interferon-β, GM-CSF, Interferon γ, or erythropoietin.

In some embodiments, the method further can include introducing into the cell a nucleic acid encoding a galactosyltransferase, wherein the nucleic acid encoding the galactosyltransferase includes a nucleotide sequence encoding a targeting sequence to target the encoded galactosyltransferase to the Golgi apparatus, wherein expression of the galactosyltransferase in the fungal cell produces the target protein modified to contain

$GalGlcNAcMan_3GlcNAc_2$ N-glycans. The target protein modified to contain $GalGlcNAcMan_3GlcNAc_2$ N-glycans can be isolated from the fungal cell.

This document also features a method of making a fungal cell (e.g., *Yarrowia lipolytica* or *Arxula adeninivorans*) capable of producing proteins containing $GlcNAcMan_3GlcNAc_2$ N-glycans. The method includes providing a fungal cell genetically engineered to produce proteins containing $Man_3GlcNAc_2$ N-glycans; introducing into the cell a nucleic acid encoding a GlcNAc-transferase I, wherein the nucleic acid includes a nucleotide sequence encoding a targeting sequence to target the encoded GlcNAc-transferase I to an intracellular compartment (e.g., Golgi apparatus), wherein expression of the GlcNAc-transferase I in the fungal cell produces proteins containing $GlcNAcMan_3GlcNAc_2$ N-glycans.

In one aspect, there is provided a method of making a *Yarrowia lipolytica* cell for producing proteins comprising $GlcNAcMan_3GlcNAc_2$ N-glycans, said method comprising:

a) providing a *Yarrowia lipolytica* cell genetically engineered to produce proteins comprising $Man_3GlcNAc_2$ N-glycans;

b) introducing into said cell a nucleic acid encoding a GlcNAc-transferase I, wherein said nucleic acid comprises a nucleotide sequence encoding a targeting sequence to target said encoded GlcNAc-transferase I to an intracellular compartment, wherein expression of said GlcNAc-transferase I in said *Yarrowia lipolytica* cell produces proteins comprising $GlcNAcMan_3GlcNAc_2$ N-glycans.

The method further can include introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein modified to contain $GlcNAcMan_3GlcNAc_2$ N-glycans. The target protein can bind to an Fc receptor. The target protein can be an antibody or fragment thereof. The target protein can be a therapeutic glycoprotein. The target protein can be Interferon-β, GM-CSF, Interferon γ, or erythropoietin.

The fungal cell genetically engineered to produce proteins containing $Man_3GlcNAc_2$ N-glycans can be deficient in ALG3 activity, and include a nucleic acid encoding an α-1,2-mannosidase, wherein the nucleic acid includes a nucleotide sequence encoding a targeting sequence to target the encoded α-1,2-mannosidase to the

endoplasmic reticulum. Such a fungal cell further can be deficient in OCH1 activity and/or further include a nucleic acid encoding α-1,3-glucosyltransferase (e.g., ALG6).

The method further can include introducing into the cell a nucleic acid encoding a GlcNAc-transferase II, wherein the nucleic acid encoding the GlcNAc-transferase II includes a nucleotide sequence encoding a targeting sequence to target the encoded GlcNAc-transferase II to an intracellular compartment, wherein expression of the GlcNAc-transferase II in the fungal cell produces proteins containing $GlcNAc_2Man_3GlcNAc_2$ N-glycans.

The method further can include introducing into the cell a nucleic acid encoding a galactosyltransferase, wherein the nucleic acid encoding the galactosyltransferase includes a nucleotide sequence encoding a targeting sequence to target the encoded

galactosyltransferase to the Golgi apparatus, wherein expression of the galactosyltransferase in the fungal cell produces proteins containing $GalGlcNAcMan_3GlcNAc_2$ or $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans. The galactosyltransferase can be a fusion of a UDP-Glc-4-epimerase and catalytic domain of a β-1,4-galactosyltransferase I. The method further can include introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein modified to contain $GalGlcNAcMan_3GlcNAc_2$ or $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

The method further can include introducing into the cell a nucleic acid encoding the α and β subunits of a Glucosidase II, wherein expression of the α and β subunits of the Glucosidase II in the fungal cell produces proteins including $GalGlcNAcMan_3GlcNAc_2$ or $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

This document also features a method of producing a target protein containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans. The method includes providing a fungal cell genetically engineered to be deficient in ALG3 activity and including a nucleic acid encoding a GlcNAc-transferase I, a GlcNAc-transferase II, and a galactosyltransferase, wherein the nucleic acid encoding the GlcNAc-transferase I, the GlcNAc-transferase II, and the galactosyltransferase include a nucleotide sequence encoding a targeting sequence, or nucleotide sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment (e.g., the Golgi apparatus); and introducing into the cell a nucleic acid encoding a target protein, wherein the cell produces the target protein containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In one aspect, there is provided a method of producing a target protein comprising $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans, said method comprising:

a) providing a *Yarrowia lipolytica* cell genetically engineered to be deficient in ALG3 activity and comprising a nucleic acid encoding a GlcNAc-transferase I, a GlcNAc-transferase II, and a galactosyltransferase, wherein said nucleic acid encoding said GlcNAc-transferase I, said GlcNAc-transferase II, and said galactosyltransferase comprises nucleotide sequences encoding targeting sequences to target each encoded protein to an intracellular compartment;

b) introducing into said cell a nucleic acid encoding a target protein, wherein said cell produces said target protein comprising said $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

The fungal cell can be further deficient in OCH1 activity and/or further include a nucleic acid encoding an α-1,3-glucosyltransferase such as ALG6. The fungal cell further can include a nucleic acid encoding the α and β subunits of a Glucosidase II, wherein expression of the α and β subunits of the Glucosidase II in the fungal cell produces the target protein containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

Further featured is~~In another aspect, this document features~~ an isolated fungal cell genetically engineered to produce proteins containing $GlcNAcMan_3GlcNAc_2$ N-glycans. The fungal cell can be deficient in OCH1 activity and include a nucleic acid encoding an α-1,2-mannosidase, a GlcNAc-transferase I, and a mannosidase II, wherein the nucleic acid encoding the α-1,2-mannosidase, the GlcNAc-transferase I, and the mannosidase II

includes a nucleotide sequence encoding a targeting sequence, or nucleotide sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment, wherein expression of the α-1,2-mannosidase, the GlcNAc-transferase I, and the mannosidase II in the fungal cell produces proteins containing $GlcNAcMan_3GlcNAc_2$ N-glycans.

In one aspect, there is provided an isolated *Yarrowia lipolytica* cell genetically engineered to produce proteins comprising $GlcNAcMan_3GlcNAc_2$ N-glycans, wherein said *Yarrowia lipolytica* cell is deficient in OCH1 activity and comprises a nucleic acid encoding an α-1,2-mannosidase, a GlcNAc-transferase I, and a mannosidase II, wherein said nucleic acid encoding said α-1,2-mannosidase, said GlcNAc-transferase I, and said mannosidase II comprises nucleotide sequences encoding targeting sequences to target each encoded protein to an intracellular compartment, wherein expression of said α-1,2-mannosidase, said GlcNAc-transferase I, and said mannosidase II in said *Yarrowia lipolytica* cell produces proteins comprising $GlcNAcMan_3GlcNAc_2$ N-glycans.

The fungal cell further can include a nucleic acid encoding a target protein, wherein the cell produces the target protein modified to contain $GlcNAcMan_3GlcNAc_2$ N-glycans.

In some embodiments, such a fungal cell further includes a nucleic acid encoding a GlcNAc-transferase II, wherein the nucleic acid encoding the GlcNAc-transferase II includes a nucleotide sequence encoding a targeting sequence to target the encoded GlcNAc-transferase II to an intracellular compartment, wherein expression of the GlcNAc-transferase II in the fungal cell produces proteins containing $GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In some embodiments, such a fungal cell further includes a nucleic acid encoding a galactosyltransferase, wherein the nucleic acid encoding the galactosyltransferase includes a nucleotide sequence encoding a targeting sequence to target the encoded galactosyltransferase to the Golgi apparatus, wherein expression of the galactosyltransferase in the fungal cell produces proteins containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

Also featured is~~In yet another aspect, this document features~~ an isolated fungal cell genetically

engineered to produce proteins containing $GlcNAc_2Man_3GlcNAc_2$ N-glycans. The fungal cell is genetically engineered to be deficient in ALG3 activity and includes a nucleic acid encoding a GlcNAc-transferase I and a GlcNAc-transferase II, wherein the nucleic acid encoding the GlcNAc-transferase I and the GlcNAc-transferase II includes a nucleotide sequence encoding a targeting sequence, or nucleotide sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment, wherein expression of the GlcNAc-transferase I, and the GlcNAc-transferase II in the fungal cell produces proteins containing $GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In one aspect, there is provided an isolated *Yarrowia lipolytica* cell genetically engineered to produce proteins comprising $GlcNAc_2Man_3GlcNAc_2$ N-glycans, wherein said *Yarrowia lipolytica* cell is genetically engineered to be deficient in ALG3 activity and comprises a nucleic acid encoding a GlcNAc-transferase I and a GlcNAc-transferase II, wherein said nucleic acid encoding said GlcNAc-transferase I and said GlcNAc-transferase II comprises nucleotide sequences encoding targeting sequences to target each encoded protein to an intracellular compartment, wherein expression of said GlcNAc-transferase I, and said GlcNAc-transferase II in said *Yarrowia lipolytica* cell produces proteins comprising $GlcNAc_2Man_3GlcNAc_2$ N-glycans.

The genetically engineered fungal cell further can be deficient in OCH1 activity and/or further include a nucleic acid encoding an α-1,3-glucosyltransferase. A genetically engineered fungal cell also can include a nucleic acid encoding a target protein, wherein the cell produces the

target protein modified to contain $GlcNAc_2Man_3GlcNAc_2$ N-glycans. A fungal cell further can include a nucleic acid encoding the α and β subunits of a Glucosidase II, wherein expression of the α and β subunits of the Glucosidase II in the fungal cell produces the protein containing $GlcNAc_2Man_3GlcNAc_2$ N-glycans. The fungal cell further can include a nucleic acid encoding a galactosyltransferase, wherein the nucleic acid encoding the galactosyltransferase includes a nucleotide sequence encoding a targeting sequence to target the encoded galactosyltransferase to the Golgi apparatus, wherein expression of the galactosyltransferase in the fungal cell produces proteins containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

This document also features a substantially pure culture of *Yarrowia lipolytica* cells, a substantial number of which are genetically engineered to produce glycoproteins containing $Gal_2GlcNac_2Man_3GlcNAc_2$ N-glycans. The cells are genetically engineered to be deficient in ALG3 activity and include a nucleic acid encoding a GlcNAc-transferase I, a GlcNAc-transferase II, and a galactosyltransferase, wherein the nucleic acid encoding the GlcNAc-transferase I, the GlcNAc-transferase II, and the galactosyltransferase include a nucleotide sequence encoding a targeting sequence, or nucleotides sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment, wherein expression of the GlcNAc-transferase I, the GlcNAc-transferase II, and the galactosyltransferase in the cell produces proteins containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In one aspect, there is provided a substantially pure culture of *Yarrowia lipolytica* cells, a substantial number of which are genetically engineered to produce glycoproteins comprising $Gal_2GlcNac_2Man_3GlcNAc_2$ N-glycans, wherein said cells are genetically engineered to be deficient in ALG3 activity and comprise a nucleic acid encoding a GlcNAc-transferase I, a GlcNAc-transferase II, and a galactosyltransferase, wherein said nucleic acid encoding said GlcNAc-transferase I, said GlcNAc-transferase II, and said galactosyltransferase comprises nucleotide sequences encoding targeting sequences to target each encoded protein to an intracellular compartment, wherein expression of said GlcNAc-transferase I, said GlcNAc-transferase II, and said galactosyltransferase in said cell produces proteins comprising $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

The genetically engineered fungal cell further can be deficient in OCH1 activity and/or further include a nucleic acid encoding an α-1,3-glucosyltransferase (e.g., ALG6). The cells further can include a nucleic acid encoding the α and β subunits of a Glucosidase II, wherein expression of the α and β subunits of the Glucosidase II in the fungal cell produces the target protein containing $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In another aspect, there is provided~~this document features~~ a substantially pure culture of *Yarrowia lipolytica* cells, a substantial number of which are genetically engineered to produce glycoproteins comprising~~containing~~ $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans, wherein the cells are genetically engineered to be deficient in OCH1 activity and comprise~~include~~ a nucleic acid encoding an α-1,2-mannosidase, a GlcNAc-transferase I, a mannosidase II, a GlcNAc-transferase II, and a galactosyltransferase, wherein the nucleic acid encoding the α-1,2-

mannosidase, the GlcNAc-transferase I, the mannosidase II, the GlcNAc-transferase II, and the galactosyltransferase comprises~~includes a nucleotide sequence encoding a targeting sequence, or~~ nucleotide sequences encoding targeting sequences, to target each encoded protein to an intracellular compartment, wherein expression of the α-1,2-mannosidase, GlcNAc-transferase I, mannosidase II, GlcNAc-transferase II, and galactosyltransferase in the cells produces proteins comprising $Gal_2GlcNAc_2Man_3GlcNAc_2$ N-glycans.

This document also features a composition that includes a glycoprotein, wherein at least 50% (e.g., at least 70% or at least 85%of the N-glycans on the glycoprotein are $GlcNAc_2Man_3GlcNAc_2$ N-glycans.

In one aspect, there is provided a composition comprising a glycoprotein, wherein at least 50% of the N-glycans on said glycoprotein are $GlcNAc_2Man_3GlcNAc_2$ N-glycans; and wherein the glycoprotein has been produced using a genetically engineered *Yarrowia lipolytica* cell.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the exemplary methods and materials are described below. All publications, patent applications, patents, Genbank® Accession Nos, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present application, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1A is a representation of $Man_5GLcNAc_2$ and $Man_3GlcNAc_2$ structures.

FIG. 1B is a schematic diagram of plasmid pYlOCH1 PUT TOPO.

FIG. 2 is a series of electroferograms depicting N-glycan analysis of secreted proteins obtained from po1d lnuga *Yarrowia lipolytica* wild-type cells or Δoch1 po1d lnuga *Yarrowia lipolytica* cells. The main N-glycan upon OCH1 inactivation becomes $Man_8GlcNAc_2$. Analysis was performed using DNA sequencer-assisted, fluorophore-assisted carbohydrate electrophoresis (DSA-FACE). "M5," "M6," "M8," and "M9," refer to the number of mannose residues conjugated to the base N-acetylglucosamine structure. The Y-axis represents the relative fluorescence units as an indication of the