In one aspect, the present disclosure provides a cell comprising: a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS comprises at least a first heme responsive motif (HRM), and wherein the ALAS comprises a mutation in the first HRM; and a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a heme-binding protein, wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding the heme-binding protein is operably linked to the first promoter element or is operably linked to a second promoter element.

In one example, provided herein is a methylotrophic yeast cell including a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS includes a first mutation in a first heme responsive motif (HRM) and a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a heme-binding protein, wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding the heme-binding protein is operably linked to the first promoter element or is operably linked to a second promoter element.

Implementations can have one or more of the following features. The methylotrophic yeast cell can be a *Pichia* cell, a *Candida* cell, a *Hansenula* cell, or a *Torulopsis* cell. The methylotrophic yeast cell can be a *Pichia methanolica* cell, a *Pichia pastoris* cell, a *Candida boidinii* cell, or a *Hansenula polymorpha* cell. The methylotrophic yeast cell can be a *Pichia pastoris* cell. The first mutation can be a cysteine to serine mutation. The first mutation can be a cysteine to alanine mutation. The ALAS can include a second mutation in a second HRM. The second mutation can be a cysteine to serine mutation. The second mutation can be a cysteine to alanine mutation. The first exogenous nucleic acid construct can include a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence in SEQ ID NO: 28. The first exogenous nucleic acid construct can include a nucleic acid sequence having at least 95% sequence identity to the nucleic acid sequence in SEQ ID NO: 28. The ALAS protein can include an amino acid sequence having at least 90% sequence identity to the amino acid sequence in SEQ ID NO: 29. The ALAS protein can include an amino acid sequence having at least 95% sequence identity to the amino acid sequence in SEQ ID NO: 29. The heme-binding protein can be selected from the group consisting of a globin, a cytochrome, a cytochrome c oxidase, a ligninase, a catalase, and a peroxidase. The heme-binding protein can be selected from the group consisting of an androglobin, a chlorocruorin, a cytoglobin, an erythrocruorin, a flavohemoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a histoglobin, a leghemoglobin, a myoglobin, a neuroglobin, a non-symbiotic hemoglobin, a protoglobin, and a truncated hemoglobin. The heme-binding protein can be a non-symbiotic hemoglobin. The heme-binding protein can be a leghemoglobin. The heme-binding protein can include an amino acid sequence having at least 90% sequence identity to an amino acid sequence in any one of SEQ ID NOs: 1-27. The methylotrophic yeast cell can further include a third nucleic acid construct comprising a nucleotide sequence

encoding a transcription factor, wherein the third nucleic acid construct is operably linked to the first promoter element, the second promoter element, or a third promoter element. The first promoter element can include a recognition sequence for the transcription factor. The second exogenous nucleic acid construct can be operably linked to a second promoter element, and wherein the second promoter element includes a recognition sequence for the transcription factor. The third nucleic acid construct can be operably linked to the third promoter element, and wherein the third promoter element includes a recognition sequence for the transcription factor. The methylotrophic yeast cell can further include a fourth nucleic acid construct comprising a nucleotide sequence encoding a protein involved in heme biosynthesis, wherein the fourth nucleic acid construct is operably linked to the first promoter element, the second promoter element, the third promoter element, or a fourth promoter element. The protein involved in heme biosynthesis can be selected from the group consisting of ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and ferrochelatase. The first exogenous nucleic acid construct can be a heterologous nucleic acid construct. The second exogenous nucleic acid construct can be a heterologous nucleic acid construct. The heme-binding protein can be an exogenous heme-binding protein. The heme-binding protein can be a heterologous heme-binding protein.

In one aspect, the present disclosure provides a method of producing a heme-binding protein in a cell comprising: expressing a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS comprises at least a first heme responsive motif (HRM), and wherein the ALAS comprises a mutation in the first HRM; and expressing a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a heme-binding protein, wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding the heme-binding protein is operably linked to the first promoter element or is operably linked to a second promoter element.

In another example, provided herein is a method of producing a heme-binding protein in a methylotrophic yeast cell including expressing a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS includes a first mutation in a first heme responsive motif (HRM) and expressing a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a heme-binding protein,
wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding the heme-binding protein is operably linked to the first promoter element or is operably linked to a second promoter element.

Implementations can have one or more of the following features. The ALAS can include a second mutation in a second HRM. The method can produce the heme-binding protein in a titer that is at least 5% greater than a corresponding method lacking the first exogenous nucleic acid construct. The method can produce the heme-binding protein in a titer that is at least 10% greater than a corresponding method lacking the first exogenous nucleic

cytochrome, a cytochrome c oxidase, a ligninase, a catalase, and a peroxidase. The heme-binding protein can be selected from the group consisting of an androglobin, a chlorocruorin, a cytoglobin, an erythrocruorin, a flavohemoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a histoglobin, a leghemoglobin, a myoglobin, a neuroglobin, a non-symbiotic hemoglobin, a protoglobin, and a truncated hemoglobin. The heme-binding protein can be a non-symbiotic hemoglobin. The heme-binding protein can be a leghemoglobin. The heterologous heme-binding protein can include an amino acid sequence having at least 90% sequence identity to an amino acid sequence in any one of SEQ ID NOs: 1-27. The method can further include expressing a third nucleic acid construct comprising a nucleotide sequence encoding a transcription factor, wherein the third nucleic acid construct is operably linked to the first promoter element, the second promoter element, or a third promoter element. The first promoter element can include a recognition sequence for the transcription factor. The second exogenous nucleic acid construct can be operably linked to a second promoter element, and wherein the second promoter element includes a recognition sequence for the transcription factor. The third nucleic acid construct can be operably linked to the third promoter element, and wherein the third promoter element includes a recognition sequence for the transcription factor. The method can further include expressing a fourth nucleic acid construct comprising a nucleotide sequence encoding a protein involved in heme biosynthesis, wherein the fourth nucleic acid construct is operably linked to the first promoter element, the second promoter element, the third promoter element, or a fourth promoter element. The protein involved in heme biosynthesis can be selected from the group consisting of ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and ferrochelatase. The first exogenous nucleic acid construct can be a heterologous nucleic acid construct. The second exogenous nucleic acid construct can be a heterologous nucleic acid construct. The heme-binding protein can be an exogenous heme-binding protein. The heme-binding protein can be a heterologous heme-binding protein.

In another example, provided herein is a *Pichia pastoris* cell including a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS includes a first mutation in a first heme responsive motif (HRM) and a second mutation in a second HRM and a second exogenous nucleic acid construct comprising a nucleotide

sequence encoding leghemoglobin, wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding leghemoglobin is operably linked to the first promoter element, or the second exogenous nucleic acid construct comprising a nucleotide sequence encoding leghemoglobin is operably linked to a second promoter element.

Implementations can include one or more of the following features. The first mutation can be a cysteine to serine mutation. The second mutation can be a cysteine to serine mutation.

In another example, provided herein is a method of producing leghemoglobin, the method including expressing a first exogenous nucleic acid construct comprising a nucleotide sequence encoding an aminolevulinate synthase (ALAS) protein operably linked to a first promoter element, wherein the ALAS includes a first mutation in a first heme responsive motif (HRM) and a second mutation in a second HRM and expressing a second exogenous nucleic acid construct comprising a nucleotide sequence encoding leghemoglobin, wherein the second exogenous nucleic acid construct comprising a nucleotide sequence encoding leghemoglobin is operably linked to the first promoter element, or the second exogenous nucleic acid construct comprising a nucleotide sequence encoding leghemoglobin is operably linked to a second promoter element.

Implementations can have one or more of the following features. The method can produce the leghemoglobin in a titer that is at least 5% greater than a corresponding method lacking the first exogenous nucleic acid construct. The method can produce the leghemoglobin in a titer that is at least 10% greater than a corresponding method lacking the first exogenous nucleic acid construct. The method can produce the leghemoglobin in a titer that is at least 15% greater than a corresponding method lacking the first exogenous nucleic acid construct. The method can produce the leghemoglobin in a titer that is at least 20% greater than a corresponding method lacking the first exogenous nucleic acid construct. The method can produce the leghemoglobin in a titer that is at least 5% greater than a corresponding method lacking the first mutation and the second mutation. The method can produce the leghemoglobin in a titer that is at least 10% greater than a corresponding method lacking the first mutation and the second mutation. The method can produce the leghemoglobin in a titer that is at least 15% greater than a corresponding method lacking the first mutation and the second mutation. The method can produce the leghemoglobin in a titer

that is at least 20% greater than a corresponding method lacking the first mutation and the second mutation. The method can be carried out in the absence of added methanol.

In another example, provided herein is a *Pichia pastoris* cell including a first exogenous nucleic acid construct comprising a nucleotide sequence encoding a protein with at least 90% sequence identity to SEQ ID NO: 29, wherein the nucleic acid encodes a serine residue in a position corresponding to position 12 of SEQ ID NO: 29, and wherein the nucleic acid encodes a serine residue in a position corresponding to position 39 of SEQ ID NO: 29, operably linked to a first promoter element.

Implementations can include one or more of the following features. The *Pichia pastoris* cell can further include a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a protein with at least 90% sequence identity to any of SEQ ID NOs: 1-27, wherein the second exogenous nucleic acid construct is operably linked to the first promoter element, or the second exogenous nucleic acid construct is operably linked to a second promoter element. The *Pichia pastoris* cell can further include a second exogenous nucleic acid construct comprising a nucleotide sequence encoding a protein with at least 90% sequence identity to SEQ ID NO: 4, wherein the second exogenous nucleic acid construct is operably linked to the first promoter element, or the second exogenous nucleic acid construct is operably linked to a second promoter element.

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 disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more examples of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims. The word “comprising” in the claims may be replaced by “consisting essentially of” or with “consisting of,” according to standard practice in patent law.