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 protein comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 29, wherein the ALAS protein comprises at least a first heme responsive motif (HRM), ~~and wherein the ALAS comprises a mutation in the first HRM~~ wherein the first HRM comprises the amino acid sequence of SEQ ID NO:32 except for a mutation relative to the amino acid sequence of SEQ ID NO:32, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 or residue 39 of SEQ ID NO: 29, and wherein the ALAS protein catalyzes the transformation of glycine and succinyl-CoA to aminolevulinic acid; 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~~ the method comprising culturing any one of the cells disclosed herein.

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

WHAT IS CLAIMED IS:

1. 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 protein comprises an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO: 29, wherein the ALAS protein comprises at least a first heme responsive motif (HRM), ~~and wherein the ALAS comprises a mutation in the first HRM~~ wherein the first HRM comprises the amino acid sequence of SEQ ID NO:32 except for a mutation relative to the amino acid sequence of SEQ ID NO:32, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 or residue 39 of SEQ ID NO: 29, and wherein the ALAS protein catalyzes the transformation of glycine and succinyl-CoA to aminolevulinic acid; 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.

2. The cell of claim 1, wherein the cell is a fungal cell, ~~or is~~ a yeast cell, a bacterial cell, an algal cell, a plant cell, an insect cell, or a mammalian cell.

3. The cell of claim ~~2~~1, wherein ~~yeast~~ the cell is a *Pichia pastoris* cell.

~~4. The cell of any one of claims 1-3, wherein the mutation in the first HRM is a mutation from a cysteine to a different amino acid.~~

~~5. The cell of any one of claims 1-4, wherein the ALAS protein comprises a second HRM, and wherein the ALAS protein comprises a mutation in the second HRM.~~

~~6. The cell of claim 5, wherein the mutation in the second HRM is a mutation from a cysteine to a different amino acid.~~

~~7. The cell of claim 5, wherein the different amino acid is the same for the mutation in the first HRM and the mutation in the second HRM.~~

~~8. The cell of claim 5, wherein the different amino acid is not the same for the mutation in the first HRM and the mutation in the second HRM.~~

~~9~~4. The cell of any one of claims ~~4-8~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is selected from the group consisting of~~ arginine, histidine, lysine, serine, threonine, asparagine, glutamine, selenocysteine, glycine, proline, alanine, isoleucine, leucine, methionine, glutamic acid, aspartic acid, phenylalanine, tryptophan, tyrosine, ~~and~~ or valine.

~~10~~5. The cell of any one of claims ~~4-8~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is selected from~~ a nonpolar aliphatic amino acid, an aromatic amino acid, a polar uncharged amino acid, or a positively charged amino acid.

~~11~~6. The cell of any one of claims ~~4-9~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is~~ serine.

~~12~~7. The cell of any one of claims ~~4-9~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is~~ alanine.

~~13~~8. The cell of any one of claims ~~4-9~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is~~ phenylalanine.

~~14~~9. The cell of any one of claims ~~4-9~~1-3, wherein the mutation of residue 12 or residue 39 of SEQ ID NO:29 is to ~~different amino acid is~~ histidine.

10. The cell of any one of claims 1-9, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 of SEQ ID NO: 29.

11. The cell of any one of claims 1-9, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29.

12. The cell of claim 10, wherein the ALAS protein comprises a second HRM, wherein the second HRM comprises the amino acid sequence of SEQ ID NO:32 except for a mutation relative to the amino acid sequence of SEQ ID NO:32, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29.

13. The cell of claim 12, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM are the same mutation.

14. The cell of claim 12, wherein the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM are not the same mutation.

15. The cell of claim 12, wherein:

(a) the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 of SEQ ID NO: 29 to serine, and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29 to serine;

(b) the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 of SEQ ID NO: 29 to alanine, and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29 to alanine;

(c) the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 of SEQ ID NO: 29 to phenylalanine, and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29 to phenylalanine; or

(d) the mutation relative to the amino acid sequence of SEQ ID NO:32 in the first HRM corresponds to a mutation of residue 12 of SEQ ID NO: 29 to histidine, and the mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM corresponds to a mutation of residue 39 of SEQ ID NO: 29 to histidine.

16. The cell of any one of claims 1-12, wherein the first HRM has the amino acid sequence of any one of SEQ ID NOs: 33-36 except for a mutation at residue 2 of any one of SEQ ID NOs:33-36, respectively.

17. The cell of claim 16, wherein the second HRM has the amino acid sequence of any one of SEQ ID NOs: 33-36 except for a mutation at residue 2 of any one of SEQ ID NOs:33-36, respectively.

~~15~~18. The cell of any one of claims 1-~~14~~17, wherein the first exogenous nucleic acid construct comprises a ~~nucleic acid~~nucleotide sequence having at least 90% sequence identity to the nucleotide~~nucleic acid~~ sequence ~~in~~ of SEQ ID NO: 28.

~~16~~19. The cell of any one of claims 1-~~15~~18, wherein the ALAS protein comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence ~~in~~ of SEQ ID NO: 29.

~~17~~20. The cell of any one of claims 1-~~16~~19, wherein the heme-binding protein is selected from the group consisting of a globin, a cytochrome, a cytochrome c oxidase, a ligninase, a catalase, and a peroxidase.

~~18~~21. The cell of any one of claims 1-~~16~~19, wherein the heme-binding protein is a leghemoglobin.

~~19~~22. The cell of any one of claims 1-~~16~~19, wherein the heme-binding protein comprises an amino acid sequence having at least 90% sequence identity to ~~an~~ the amino acid sequence ~~in~~ of any one of SEQ ID NOs: 1-27.

~~20~~23. A method of producing a heme-binding protein, ~~in a cell~~ the method comprising culturing the cell of any one of claims 1-22.~~:~~

~~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.~~

~~21~~24. The method of ~~20~~23, wherein the method produces the heme-binding protein ~~in~~ at a titer that is at least 5% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and lacking the first exogenous nucleic acid construct.

~~22~~25. The method of claim ~~20~~ 23 or claim ~~21~~24, wherein the method produces the heme-binding protein ~~in~~ at a titer that is at least 10% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and lacking the first exogenous nucleic acid construct.

~~23~~26. The method of claim ~~20~~23, wherein the method produces the heme-binding protein ~~in~~ at a titer that is at least 5% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and the first exogenous nucleic acid construct, except wherein the first exogenous nucleic acid

construct in the cell of the corresponding method lacks a~~lacking the~~ mutation in the first HRM.

~~24~~27. The method of claim ~~20~~23, wherein the method produces the heme-binding protein ~~in~~ at a titer that is at least 10% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and the first exogenous nucleic acid construct, except wherein the first exogenous nucleic acid construct in the cell of the corresponding method lacks a~~lacking the~~ mutation in the first HRM.

~~25~~28. The method of any one of claims ~~20-24~~23-27, wherein the method is carried out in the absence of ~~added~~ methanol.

~~26. The method of any one of claims 20-25, wherein the mutation in the first HRM is a mutation from a cysteine to a different amino acid.~~

~~27. The method of any one of claims 20-26, wherein the ALAS protein comprises a second HRM, and wherein the ALAS protein comprises a mutation in the second HRM.~~

~~28~~29. The method of claim ~~26~~23, wherein the method produces the heme-binding protein in a titer that is at least 5% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and the first exogenous nucleic acid construct, except wherein the first exogenous nucleic acid construct in the cell of the corresponding method lacks a~~lacking the~~ mutation~~s~~ relative to the amino acid sequence of SEQ ID NO:32 in the first HRM and a mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM.

~~29~~30. The method of claim ~~26~~23, wherein the method produces the heme-binding protein in a titer that is at least 10% greater than a corresponding method comprising culturing a cell comprising the second exogenous nucleic acid construct and the first exogenous nucleic acid construct, except wherein the first exogenous nucleic acid

construct in the cell of the corresponding method lacks a~~lacking the~~ mutation~~s~~ relative to the amino acid sequence of SEQ ID NO:32 in the first HRM and a mutation relative to the amino acid sequence of SEQ ID NO:32 in the second HRM.

~~30. The method of any one of claims 27-29, wherein the mutation in the second HRM is a mutation from a cysteine to a different amino acid.~~

~~31. The method of claim 30, wherein the different amino acid is the same for the mutation in the first HRM and the mutation in the second HRM.~~

~~32. The method of claim 30, wherein the different amino acid is not the same for the mutation in the first HRM and the mutation in the second HRM.~~

~~33. The method any one of claims 26-32, wherein the different amino acid is selected from the group consisting of arginine, histidine, lysine, serine, threonine, asparagine, glutamine, selenocysteine, glycine, proline, alanine, isoleucine, leucine, methionine, aspartic acid, glutamic acid, phenylalanine, tryptophan, tyrosine, and valine.~~

~~34. The method of any one of claims 26-33, wherein the different amino acid is serine.~~

~~35. The method of any one of claims 26-33, wherein the different amino acid is alanine.~~

~~36. The method of any one of claims 26-33, wherein the different amino acid is phenylalanine.~~

~~37. The method of any one of claims 26-33, wherein the different amino acid is histidine.~~

~~38. The method of any one of claims 20-37, wherein the first exogenous nucleic acid construct comprises a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence in SEQ ID NO: 28.~~

~~39. The method of any one of claims 20-38, wherein the ALAS protein comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence in SEQ ID NO: 29.~~

~~40. The method of any one of claims 20-39, wherein the heme-binding protein is selected from the group consisting of a globin, a cytochrome, a cytochrome c oxidase, a ligninase, a catalase, and a peroxidase.~~

~~41. The method of any one of claims 20-39, wherein the heme-binding protein is a leghemoglobin.~~

~~42. The method of any one of claims 20-39, wherein the heterologous heme-binding protein comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence in any one of SEQ ID NOs: 1-27.~~