Summary

In one aspect, there is provided a genetically modified yeast, comprising:

at least one genetic modification that substantially blocks beta oxidation activity;

at least one genetic modification that increase at least one activity selected from the group consisting of: monooxygenase activity, monooxygenase reductase activity, thioesterase activity, acyltransferase activity, isocitrate dehydrogenase activity, glyceraldehyde-3-phosphate dehydrogenase activity, glucose-6-phosphate dehydrogenase activity, acyl-coA oxidase activity, fatty alcohol oxidase activity, acyl-CoA hydrolase activity, alcohol dehydrogenase activity, peroxisomal biogenesis factor activity, fatty aldehyde dehydrogenase activity, CTF, UTR, FAT1; and

at least one genetic modification that decreases MIG1 activity, as compared to corresponding yeast not comprising said genetic modification.

In one aspect, there is provided a method for producing a desired diacid, comprising:

contacting a genetically modified yeast as described herein with a feedstock capable of being converted by the yeast to a diacid; and

culturing the yeast under conditions in which the desired diacid is produced from the feedstock.

In one aspect, there is provided a method for producing a desired diacid by a yeast from a feedstock toxic to the yeast, comprising:

(a) contacting a genetically modified yeast in culture with a feedstock not substantially toxic to the yeast, thereby performing an induction; and

(b) contacting the yeast after the induction in (a) with a feedstock toxic to the yeast,

whereby the desired diacid is produced by the yeast from the feedstock toxic to the yeast in an amount greater than the amount of the diacid produced from the feedstock toxic to the yeast when the induction is not performed.

In one aspect, there is provided a diacid produced by the method as described herein.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes a polypeptide of SEQ ID NO: 148 or 150.

In one aspect, there is provided an isolated nucleic acid, comprising a polynucleotide that comprises: the nucleotide sequence of SEQ ID NO: 37 or a nucleotide sequence having greater than 75% identity to SEQ ID NO: 37.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 44; a polypeptide comprising an amino acid sequence having greater than 71% identity to SEQ ID NO: 44; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 44.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 46; a polypeptide comprising an amino acid sequence having greater than 71% identity to SEQ ID NO: 46; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 46.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 48; a polypeptide comprising an amino acid sequence having greater than 87% identity to SEQ ID NO: 48; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 48.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 50; a polypeptide comprising an amino acid sequence having greater than 80% identity to SEQ ID NO: 50; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 50.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 67; a polypeptide comprising an amino acid sequence having greater than 99% identity to SEQ ID NO: 67; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 67.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 74; a polypeptide comprising an amino acid sequence

having greater than 99% identity to SEQ ID NO: 74; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 74.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 76; a polypeptide comprising an amino acid sequence having greater than 99% identity to SEQ ID NO: 76; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 76.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 3; a polypeptide comprising an amino acid sequence having greater than 99% identity to SEQ ID NO: 3; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 3.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 5; a polypeptide comprising an amino acid sequence having greater than 99% identity to SEQ ID NO: 5; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 5.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 7; a polypeptide comprising an amino acid sequence having greater than 99% identity to SEQ ID NO: 7; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 7.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 34; a polypeptide comprising an amino acid sequence having greater than 95% identity to SEQ ID NO: 34; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 34.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 36; a polypeptide comprising an amino acid sequence having greater than 94% identity to SEQ ID NO: 36; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 36.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 129; a polypeptide comprising an amino acid sequence having greater than 89% identity to SEQ ID NO: 129; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 129.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 113; a polypeptide comprising an amino acid sequence having greater than 85% identity to SEQ ID NO: 113; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 113.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 115; a polypeptide comprising an amino acid sequence having greater than 97% identity to SEQ ID NO: 115; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 115.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 117; a polypeptide comprising an amino acid sequence having greater than 80% identity to SEQ ID NO: 117; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 117.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 119; a polypeptide comprising an amino acid sequence having greater than 84% identity to SEQ ID NO: 119; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 119.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 121; a polypeptide comprising an amino acid sequence having greater than 81% identity to SEQ ID NO: 121; or a polypeptide comprising an

amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 121.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 123; a polypeptide comprising an amino acid sequence having greater than 90% identity to SEQ ID NO: 123; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 123.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 125; a polypeptide comprising an amino acid sequence having greater than 80% identity to SEQ ID NO: 125; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 125.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 127; a polypeptide comprising an amino acid sequence having greater than 81% identity to SEQ ID NO: 127; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 127.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 96; a polypeptide comprising an amino acid sequence having greater than 85% identity to SEQ ID NO: 96; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 96.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 139; a polypeptide comprising an amino acid sequence having greater than 76% identity to SEQ ID NO: 139; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 139.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 141; a polypeptide comprising an amino acid

sequence having greater than 83% identity to SEQ ID NO: 141; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 141.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 90; a polypeptide comprising an amino acid sequence having greater than 95% identity to SEQ ID NO: 90; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO:90.

In one aspect, there is provided an isolated nucleic acid comprising a polynucleotide that encodes: the polypeptide of SEQ ID NO: 40; a polypeptide comprising an amino acid sequence having greater than 92% identity to SEQ ID NO: 40; or a polypeptide comprising an amino acid sequence having 1 to 5 amino acid substitutions in the sequence of SEQ ID NO: 40.

In one aspect, there is provided an isolated nucleic acid, comprising a polynucleotide that comprises: the nucleotide sequence of SEQ ID NO: 166 or a nucleotide sequence having greater than 84% identity to SEQ ID NO: 166.

In one aspect, there is provided an isolated nucleic acid, comprising a polynucleotide that comprises: the nucleotide sequence of SEQ ID NO: 167 or a nucleotide sequence having greater than 85% identity to SEQ ID NO: 167.

In one aspect, there is provided an isolated nucleic acid, comprising a polynucleotide that comprises: the nucleotide sequence of SEQ ID NO: 164 or a nucleotide sequence having greater than 92% identity to SEQ ID NO: 169.

In one aspect, there is provided an expression vector comprising the isolated nucleic acid as described herein.

In one aspect, there is provided a host cell comprising a nucleic acid as described herein.

In one aspect, there is provided an expression product comprising dodecanedioic acid obtained by a method as described herein.

In one aspect, there is provided an expression product comprising dodecanedioic acid obtained by contacting a genetically modified yeast as described herein with a suitable feedstock; and culturing the yeast under conditions such that said expression product comprising dodecanedioic acid is produced from said feedstock.

Provided in certain aspects is a genetically modified yeast, comprising: one or more genetic modifications that substantially block beta oxidation activity; and one or more genetic modifications that increase one or more activities chosen from monooxygenase activity, monooxygenase reductase activity, thioesterase activity, acyltransferase activity, isocitrate dehydrogenase activity, glyceraldehyde-3-phosphate dehydrogenase activity, glucose-6-phosphate dehydrogenase activity, acyl-coA oxidase activity, fatty alcohol oxidase activity, acyl-CoA hydrolase activity, alcohol dehydrogenase activity, peroxisomal biogenesis factor activity, and fatty aldehyde dehydrogenase activity.

The one or more genetic modifications sometimes increase one or more of: (a) one or more monooxygase activities chosen from monooxygenase activity chosen from CYP52A12 monooxygenase activity, CYP52A13 monooxygenase activity, CYP52A14 monooxygenase activity, CYP52A15 monooxygenase activity, CYP52A16 monooxygenase activity, CYP52A17 monooxygenase activity, CYP52A18 monooxygenase activity, CYP52A19 monooxygenase activity, CYP52A20 monooxygenase activity, CYP52D2 monooxygenase activity and BM3 monooxygenase activity; (b) one or more monooxygenase reductase activities chosen from CPRA monooxygenase reductase activity, CPRB monooxygenase reductase activity and CPR750 monooxygenase reductase activity; (c) an IDP2 isocitrate dehydrogenase activity; (d) a GDP1 glyceraldehyde-3-phosphate dehydrogenase activity; (e) one or more glucose-6-phosphate dehydrogenase activities chosen from a ZWF1 glucose-6-phosphate dehydrogenase activity and ZWF2 glucose-6-phosphate dehydrogenase activity; (f) one or more fatty alcohol oxidase activities chosen from FAO1 fatty alcohol oxidase activity, FAO2A fatty alcohol oxidase activity, FAO2B fatty alcohol oxidase activity, FAO13 fatty alcohol oxidase activity, FAO17 fatty alcohol oxidase activity, FAO18 fatty alcohol oxidase activity and FAO20 fatty alcohol oxidase activity; (g) one or more alcohol dehydrogenase activities chosen from ADH1 alcohol dehydrogenase activity, ADH2 alcohol dehydrogenase activity, ADH3 alcohol dehydrogenase activity, ADH4 alcohol dehydrogenase activity, ADH5 alcohol dehydrogenase activity, ADH7 alcohol dehydrogenase activity, ADH8 alcohol dehydrogenase activity and SFA alcohol dehydrogenase activity; (h) one or more acyl-CoA hydrolase activities chosen from

ACH-A acyl-CoA hydrolase activity and ACH-B acyl-CoA hydrolase activity; (i) one or more acyltransferase activities chosen from acyl-CoA sterol acyltransferase activity, diacylglycerol