Supporting Information

Westfall et al. 10.1073/pnas.1110740109

SI Materials and Methods

Strains and Genetic Manipulations. ***General techniques.*** *Saccharomyces cerevisiae* strains (Table S1) were grown on synthetic complete medium (1) agar plates at 30 °C with 2% glucose as the carbon source unless otherwise indicated, lacking leucine where needed for selection of plasmids. Antibiotics [Geneticin (2), Hygromycin B (3), and nourseothricin (3)] or 5-fluoroorotic acid (4), were added as necessary to select for integrative transformants. Standard DNA manipulation (5, 6) and yeast genetic (1) techniques were used.

Synthetic genes. *S. cerevisiae* codon-optimized synthetic genes of *AaADS* (GenBank JF951730), *CYP71AV1* (GenBank JF951731), and *AaCPR* (GenBank JF951732) were synthesized by DNA 2.0 (https://www.dna20.com/) or Biosearch Technologies, Inc.

Construction of genome integration cassettes. Oligonucleotide primers are listed in Table S2.

gal1Δ::K. lactis URA3. To render galactose a gratuitous inducer in the EPY330 background, primers 50-10-pw58-G and 50-10-pw58-G were used to amplify the *K. lactis URA3* marker which has been previously cloned into the TOPO TA vector (Invitrogen). Positive transformants were selected for their ability to grow on medium lacking uracil and further confirmed by diagnostic PCR and their inability to grow on medium containing galactose as a sole carbon source.

Construction of CEN.PK2 Gen 1.0. The base strains for both Gen 1.0 and Gen 2.0 constructions were CEN.PK2-1C and CEN.PK2-1D [EUROSCARF (http://web.uni-frankfurt.de/fb15/mikro/euroscarf/), accession no. 30000A; *MATa; ura3-52; trp1-289; leu2-3,112; his3Δ1; MAL2-8C; SUC2*]. Template genomic DNA for PCR reactions was isolated using the Y-DER yeast DNA extraction kit (Pierce Biotechnologies). PCR amplification used Phusion High Fidelity DNA Polymerase (Finnzymes OY).

erg9::Kan^r-P$_{MET3}$-ERG9.
Primary PCR amplification of the kanamycin resistance marker was performed with oligonucleotides 50-3-pw1-G and 50-3-pw2-G from pFA6-KanMX-PGAL1 (7). Primary PCR amplification of the *MET3* promoter was performed with oligonucleotides 50-3-pw3-G and 50-27-pw80-G from pESC-ERG9 (8) template. Secondary PCR using the KanMX and P_{MET3} amplicons as template was performed with oligonucleotides 50-3-pw1-G and 50-27-pw80-G. The *kanMX-P$_{MET3}$PCR* product was ligated into the TOPO ZERO Blunt II Cloning vector (Invitrogen) and given the designation pAM328.

trp1::TRP1-P$_{GAL1}$-ERG20.
The *TRP1-P$_{GAL1}$* region of plasmid *pFA6a-TRP1-P$_{GAL1}$* (7) was amplified with oligonucleotide 50-18-pw64-G and 50-18-pw65-G. *ERG20* was amplified from BY4710 (9) genomic DNA with oligonucleotide 50-18-pw66-G and 50-18-pw67-G. Secondary PCR was performed with the *TRP1-P$_{GAL1}$* and *ERG20* amplicons using oligonucleotides 50-18-pw64-G and 50-18-pw67-G. The resulting *TRP1-P$_{GAL1}$-ERG20* PCR product was ligated into the TOPO ZERO Blunt II Cloning vector and given the designation pAM329.

his3::HIS-P$_{GAL1}$-tHMG1 and leu2::HIS-P$_{GAL1}$-tHMG1.
The *His3MX6-P$_{GAL1}$* region of plasmid *pFA6a-His3MX-P$_{GAL1}$* was amplified with oligonucleotide 50-71-pw111-G and 50-72-pw112-G. *tHMGR* was amplified using BY4710 as the template with oligonucleotide 50-71-pw113-G and 50-71-pw114-G. Secondary PCR was performed with the *His3MX6-P$_{GAL1}$* and *tHMGR1* amplicons using oligonucleotides 50-71-pw111-G and 50-71-pw114-G. The *HisMX6-P$_{GAL1}$-tHMGR* PCR product was ligated into the TOPO ZERO Blunt II Cloning vector and given the designation pAM330.

ura3::URA3-P$_{GAL1}$-UPC2-1.
URA3 was amplified from BY4710 genomic DNA with oligonucleotides 50-104-pw176-G and 50-113-pw201-G. *P$_{GAL1}$-UPC2-1* was amplified from EPY300 (10) genomic DNA with oligonucleotides 50-113-pw199-G and 50-113-pw200-G. Secondary PCR was performed with the *URA3* and *P$_{GAL1}$-upc2-1* amplicons using oligonucleotides 50–104-pw176-G and 50–113-pw199-G. The *URA3-P$_{GAL1}$-upc2-1* PCR product was ligated into the TOPO ZERO Blunt II Cloning vector and given the designation pAM333.

Creation of Y135.
Replacement of the native *ERG9* promoter with the repressible *MET3* promoter was done by amplifying the *kanMX6-P$_{MET3}$* region of pAM328 with oligonucleotides 50-56-pw100-G and 50-56-pw101-G containing approximately 50 nt of flanking homology to the *ERG9* promoter region. The resulting PCR amplicon was transformed into CEN.PK2-1C and CEN.PK2-1D. Correct transformants were selected for growth in the presence of Geneticin (Invitrogen) and confirmed by diagnostic PCR. The resultant clones were given the designations Y93 and Y94.

The *trp1-289* loci of Y93 and Y94 were replaced by amplifying the *TRP1-P$_{GAL1}$-ERG20* region from pAM329 with oligonucleotides 50-18-pw64-G and 50-18-pw71-G. The resulting amplicon was transformed into Y93 and Y94. Correct transformants were selected for growth in the absence of tryptophan and confirmed by diagnostic PCR. The resultant clones were given the designation Y95 and Y96.

The *his3Δ1* locus of Y95 was replaced by amplifying the *hisMX-P$_{GAL1}$-tHMG1* region from pAM330 with oligonucleotides 50-100-pw162-G and 50-100-pw163-G. The resulting amplicon was transformed into Y95. Correct transformants were selected for growth in the absence of histidine and confirmed by diagnostic PCR. The resultant clone was given the designation Y97.

The *leu2-3,112* locus of Y96 was replaced by amplifying the *His3MX-P$_{GAL1}$-tHMG1* region from pAM330 with oligonucleotides 50-100-pw166-G and 61-32-pw250-G. The resulting amplicon was transformed into Y96. Correct transformants were selected for growth in the absence of leucine and confirmed by diagnostic PCR. The resultant clone was given the designation Y132.

The *ura3-52* locus of Y97 was replaced by amplifying the *URA3-P$_{GAL1}$-UPC2-1* region from pAM333 with oligonucleotides 50-104-pw176-G and 61-13-pw242-G. The resulting amplicon was transformed into Y108. Correct transformants were selected for growth in the absence of uracil and confirmed by diagnostic PCR. The resultant clone was given the designation Y131.

Approximately 1×10^7 cells from Y131 and Y132 were mixed on a YPD medium agar plate for 6 h at room temperature to allow for mating. Fused diploid cells (zygotes) were identified by their distinctive barbell shape and isolated using a Singer Instruments MSM300 series micromanipulator (Singer Instrument Co, LTD). Diploid cells containing both *MATa* and *MAT*α mating types were confirmed by diagnostic PCR (11).

To generate haploid strains containing the full complement of introduced genes, the diploid strains described above were sporulated in 2% potassium acetate and 0.02% raffinose liquid medium. Twenty tetrads were dissected using a Singer Instruments MSM300 series micromanipulator. Independent genetic isolates containing the appropriate complement of introduced genetic material were identified by their ability to grow in the absence of histidine and uracil and confirmed by diagnostic PCR. A single *MAT*α isolate containing all introduced constructs was transformed with plasmid pAM322. The resultant transformant was designated Y135.

Construction of CEN.PK2 Gen 2.0.

erg9Δ::*kan^r_P_MET3-ERG9*.
Same as CEN.PK2 Gen 1.0.

leu2-3,112::*HIS_P_GAL1-ERG19_P_GAL10-ERG8*.
For targeted integration into the *S. cerevisiae leu2-3,112* locus, primary amplifications of the wild-type *LEU2* locus were performed using BY4710 (9) genomic DNA as template, as follows: nucleotides 100–450 were PCR amplified with oligonucleotides 61-67-CPK019-G and 61-67-CPK020-G, and from nucleotides 1096 to 1770 with oligonucleotides 61-67-CPK023-G and 61-67-CPK024-G. *HIS* was PCR amplified with oligonucleotides 61-67-CPK021-G and 61-67-CPK022-G. For the secondary PCR, the $LEU2^{1096\text{ to }1770}$ and *HIS* amplicons were used as the DNA template and PCR amplified with oligonucleotides 61-67-CPK021-G and 61-67-CPK024-G to give $HIS\text{-}LEU2^{1096\text{ to }1770}$. The tertiary PCR reaction, containing $LEU2^{-100\text{ to }450}$ and $HIS\text{-}LEU2^{1096\text{ to }1770}$ as template, was performed with oligonucleotides 61-67-CPK019-G and 61-67-CPK024-G to generate $LEU2^{-100\text{ to }450}\text{-}HIS\text{-}LEU2^{1096\text{ to }1770}$ flanked by PmeI restriction sites and containing an internal XmaI restriction site between the *HIS* and $LEU2^{1096\text{ to }1770}$. This product was ligated into the TOPO pCR2.1 cloning vector and the resulting plasmid given the designation pAM470. *ERG8* and $P_{GAL1,10}$ were PCR amplified from CEN-PK2-1c genomic DNA using oligonucleotides 61-67-CPK041-G and 61-67-CPK060-G, and 61-67-CPK061-G and 61-67-CPK062-G, respectively. *ERG19* was PCR amplified with oligonucleotides 61-67-CPK046-G and 61-67-CPK063-G. Secondary PCR using *ERG8* and $P_{GAL1,10}$ amplicons as the DNA template was performed with oligonucleotides 61-67-CPK041-G and 61-67-CPK062-G to give $ERG8\text{-}P_{GAL1,10}$. Tertiary PCR using $ERG8\text{-}P_{GAL1,10}$ and *ERG19* amplicons as the DNA template was performed with oligonucleotides 61-67-CPK041-G and 61-67-CPK046-G to give $ERG8\text{-}P_{GAL1,10}\text{-}ERG19$. The $ERG8\text{-}P_{GAL}\text{-}ERG19$ PCR product was ligated into the TOPO ZERO Blunt II Cloning vector (Invitrogen) and given the designation pAM475. pAM497 was generated by isolation of the $ERG8\text{-}P_{GAL1,10}\text{-}ERG19$ insert from pAM475 following XmaI digestion, and its ligation into XmaI digested pAM470. The $LEU2^{100\text{ to }450}\text{-}HIS\text{-}ERG19\text{-}P_{GAL1,10}\text{-}ERG8\text{-}LEU2^{1096\text{ to }1770}$ insert in pAM497 was verified by DNA sequencing. The final PCR amplicon containing $HIS3^{-32\text{ to }-1000}\text{-}HISMX\text{-}HIS3^{504\text{ to }-1103}$ flanked by PmeI restriction sites and containing an internal XmaI restriction site between the *HIS* and $HIS3^{504\text{ to }-1103}$ was ligated into the TOPO pCR2.1 cloning vector. The resulting plasmid was given the designation pAM469.

his3Δ1::*HIS_P_GAL1-ERG12_P_GAL10-ERG10*.
For targeted integration into the *S. cerevisiae his3Δ1*locus, primary amplifications of the wild-type *HIS3* locus were performed using BY4710 (9) genomic DNA as template, as follows: nucleotides −32 to −1000 and 504 to 1103 were PCR amplified with oligonucleotides 61-67-CPK013-G and 61-67-CPK014alt-G, and 61-67-CPK017-G and 61-67-CPK018-G, respectively. *HIS* was PCR amplified with oligonucleotides 61-67-CPK015alt-G and 61-67-CPK016-G. For the secondary PCR, 100 ng each of the purified $HIS3^{504\text{ to }-1103}$ and *HIS* amplicons were used as the DNA template and PCR amplified with oligonucleotides 61-67-CPK015alt-G and 61-67-CPK018-G to give $HIS\text{-}HIS3^{504\text{ to }-1103}$. The tertiary PCR reaction with $HIS3^{-32\text{ to }-1000}$ and $HIS\text{-}HIS3^{504\text{ to }1103}$ amplicons as template was performed with oligonucleotides 61-67-CPK013-G and 61-67-CPK018-G. The final PCR fragment containing $HIS3^{-32\text{ to }-1000}\text{-}HIS\text{-}HIS3^{504\text{ to }-1103}$ flanked by PmeI restriction sites and containing an internal XmaI restriction site between the HIS and $HIS3^{504\text{ to }-1103}$ was ligated into the TOPO pCR2.1 cloning vector. The resulting plasmid was given the designation pAM469. *ERG10* and *ERG12* were PCR amplified from CEN.PK2-1c genomic DNA template with oligonucleotides 61-67-CPK035-G and 61-67-CPK056-G, and 61-67-CPK040-G and 61-67-CPK059-G, respectively. $P_{GAL1,10}$ was PCR amplified using oligonucleotides 61-67-CPK057-G and 61-67-CPK058-G. Secondary PCR using the *ERG10* and $P_{GAL1,10}$ amplicons as DNA template was performed with oligonucleotides 61-67-CPK035-G and 61-67-CPK058-G to give $ERG10\text{-}P_{GAL1,10}$. Tertiary PCR using the $ERG10\text{-}P_{GAL1,10}$ and *ERG12* PCR products as the DNA template was performed with oligonucleotides 61-67-CPK035-G and 61-67-CPK040-G to give $ERG10\text{-}P_{GAL1,10}\text{-}ERG12$. The $ERG10\text{-}P_{GAL1,10}\text{-}ERG12$ PCR product was ligated into the TOPO ZERO Blunt II Cloning vector and given the designation pAM474. pAM495 was generated by isolation of the $ERG10\text{-}P_{GAL1,10}\text{-}ERG12$ insert from pAM474 following XmaI digestion, and its ligation into XmaI digested pAM469. The $HIS3^{-32\text{ to }-1000}\text{-}HIS\text{-}ERG12\text{-}P_{GAL1,10}\text{-}ERG10\text{-}HI3^{504\text{ }-1103}$ insert in pAM495 was verified by DNA sequencing.

ade1Δ::*P_GAL1-tHMG1_P_GAL10-IDI1_ADE1*.
For targeted integration into the *S. cerevisiae ade1::LEU2* locus, primary amplifications of the wild-type *ADE1* locus were performed using BY4710 (9) genomic DNA as template, as follows: nucleotides −825 to −226 and −225 to 653 were PCR amplified with oligonucleotides 61-67-CPK009-G and 61-67-CPK0010-G, and 61-67-CPK011-G and 61-67-CPK012-G, respectively. For the secondary PCR, purified $ADE1^{-825\text{ to }-226}$ and $ADE1^{-225\text{ to }653}$ amplicons were used as the DNA template and PCR amplified with oligonucleotides 61-67-CPK009-G and 61-67-CPK012-G to give $ADE1^{-825\text{ to }653}$. The final PCR fragment containing $ADE1^{-825\text{ to }653}$ flanked with PmeI restriction sites and containing an internal XmaI site between positions −226 and −225 was ligated into the TOPO pCR2.1 cloning vector. The resulting plasmid was given the designation pAM468. *IDI1* was PCR amplified with oligonucleotides 61-67-CPK047-G and 61-67-CPK064-G; $P_{GAL1,10}$ was PCR amplified with oligonucleotides 61-67-CPK052-G and 61-67-CPK065-G; *tHMG1* was PCR amplified with oligonucleotides 61-67-CPK031-G and 61-67-CPK053-G (all from CEN.PK2-1c genomic DNA template). Secondary PCR using *IDI1* and $P_{GAL1,10}$ amplicons as the DNA template was performed with oligonucleotides 61-67-CPK047-G and 61-67-CPK052-G to give $IDI1\text{-}P_{GAL1,10}$. Tertiary PCR using the $IDI1\text{-}P_{GAL1,10}$ and *tHMG1* amplicons as the DNA template was performed with oligonucleotides 61-67-CPK031-G and 61-67-CPK047-G to give $IDI1\text{-}P_{GAL1,10}\text{-}tHMG1$. The $IDI1\text{-}P_{GAL}\text{-}tHMG1$ PCR product flanked by XmaI restriction sites was ligated into the TOPO ZERO Blunt II Cloning vector and given

the designation pAM473. pAM493 was generated by isolation of the *IDI*1-$P_{GAL1,10}$-*tHMG*1 insert from pAM473 following XmaI digestion, and its ligation into XmaI digested pAM468. The *ADE*1$^{-825\ to\ -226}$-*tHMG*1-$P_{GAL1,10}$-*IDI*1-*ADE*1$^{-225\ to\ 653}$ insert in pAM493 was verified by DNA sequencing.

***ura*3-52::P_{GAL1}-*tHMG*1_P_{GAL10}-*ERG*13_*URA*3.**
For targeted integration into the *S. cerevisiae* ura3-52 locus, primary amplifications of the wild-type *URA3* locus were performed using BY4710 (9) genomic DNA as template, as follows: nucleotides −723 to −224 were amplified by PCR using oligonucleotides 61-67-CPK005-G and 61-67-CPK006-G, and from nucleotides −223 to 701 with oligonucleotides 61-67-CPK007-G and 61-67-CPK008-G. For the secondary PCR, purified *URA*3$^{-723\ to\ -224}$ and *URA*3$^{-223\ to\ 701}$ PCR were used as the DNA template and PCR amplified with oligonucleotides 61-67-CPK005-G and 61-67-CPK008-G to give *URA*3$^{-723\ to\ 701}$. The final PCR fragment containing *URA*3$^{-723\ to\ 701}$ flanked with PmeI restriction sites and containing an internal XmaI site between positions −224 and −223 was cloned into the TOPO pCR2.1 plasmid vector. The resulting plasmid was given the designation pAM467. PCR amplification of *ERG13* was performed with oligonucleotides 61-67-CPK032-G and 61-67-CPK054-G from CEN.PK2-1c genomic DNA template. $P_{GAL1,10}$ was amplified with oligonucleotides 61-67-CPK051-G and 61-67-CPK052-G from CEN.PK2-1c genomic DNA. *tHMG1* was PCR amplified from CEN.PK2-1c genomic DNA using oligonucleotides 61-67-CPK031-G and 61-67-CPK053-G. Secondary PCR amplification used *ERG13* and $P_{GAL1,10}$ PCR products as the DNA template with oligonucleotides 61-67-CPK032-G and 61-67-CPK052-G to give *ERG*13-$P_{GAL1,10}$. Tertiary PCR of the *ERG*13-$P_{GAL1,10}$ and *tHMG1* PCR products as the DNA template was performed with oligonucleotides 61-67-CPK031-G and 61-67-CPK032-G to give *ERG*13-$P_{GAL1,10}$-*tHMGR*. The *ERG*13-$P_{GAL1,10}$-*tHMG*1 PCR product flanked by XmaI restriction sites was subcloned in the TOPO ZERO Blunt II Cloning vector and given the designation pAM472. pAM491 was generated by isolation of the *ERG*13-$P_{GAL1,10}$-*tHMG*1 insert following XmaI digestion, and its ligation into XmaI digested pAM467. The *URA*3$^{-723\ to\ -224}$-*tHMG*-$P_{GAL1,10}$-*ERG*13-*URA*3$^{-223\ to\ 701}$ insert in pAM491 was verified by DNA sequencing.

***trp*1-289::P_{GAL1}-*tHMG*1_P_{GAL10}-*ERG*20_*TRP*1.**
For targeted integration into the *S. cerevisiae trp1-289* locus, the wild-type *TRP1* locus from nucleotide position −856 to 548 (A of ATG start codon is nucleotide 1) was amplified by PCR using EPY300 (10) genomic DNA as template using overlapping primer extension such that a nonnative XmaI (CCCGGG) restriction site was created between bases −226 and −225. In addition, PmeI restriction sites (GTTTAAAC) were incorporated in the 5′ and 3′ PCR primers for simplified excision of the final PCR product from plasmid-based vectors. PCR product *TRP*1$^{-856\ to\ -226}$ was amplified with primers 61-67-CPK001-G and 61-67-CPK002-G. PCR product *TRP*1$^{-225\text{-}to\ +548}$ was amplified with primers 61-67-CPK003-G and 61-67-CPK004-G. Amplicons *TRP*1$^{-856\ to\ -226}$ and *TRP*1$^{-225\text{-}to\ +548}$ were combined and amplified in a second PCR amplification using primers 61-67-CPK001-G and 61-67-CPK004-G. The resultant *TRP*1$^{-856\ to\ +548}$ amplicon was ligated into the TOPO TA pCR2.1 cloning vector and transformed into *Escherichia coli* DH5α, generating plasmid pAM466. *ERG20* was PCR amplified with oligonucleotides 61-67-CPK025-G and 61-67-CPK050-G. $P_{GAL1,10}$ was amplified with oligonucleotides 61-67-CPK051-G and 61-67-CPK052-G from CEN.PK2-1c genomic DNA. Truncated HMG1 (*tHMG1*) was amplified with oligonucleotides 61-67-CPK053-G and 61-67-CPK031-G. The *ERG20* and $P_{GAL1,10}$ amplicons were combined and further amplified with oligonucleotides 61-67-CPK025-G and 61-67-CPK052-G. A tertiary PCR combined the resulting *ERG*20-$P_{GAL1,10}$ amplicon with the *tHMG1* using 61-67-CPK025-G and 61-67-CPK031-G to generate the *ERG*20-$P_{GAL1,10}$-*tHMG*1 amplicon, which was ligated into the TOPO Zero Blunt II cloning vector. The *ERG*20-$P_{GAL1,10}$-*tHMG*1 amplicon contained XmaI sites at either end, which were digested to release *ERG*20-$P_{GAL1,10}$-*tHMG*1; this fragment was then ligated into XmaI digested pAM466. The resulting plasmid, pAM489 contained the *TRP*1$^{-856\ to\ -226}$-*ERG*20-P_{GAL}-*tHMG*1-*TRP*1$^{-225\ to\ 548}$ insertion, which was verified by DNA sequencing.

Generation of *S. cerevisiae* galactose-inducible production strains Y224 and Y227.
The promoter of *ERG9* in CEN.PK2-1C and CEN.PK2-1D was replaced with P_{MET3} as described (12). Next, the *ADE1* open reading frame was replaced with the *Candida glabrata LEU2* gene (*CgLEU2*). The 3.5 KB *CgLEU2* genomic locus was amplified from *C. glabrata* genomic DNA (American Type Culture Collection) using primers 61-67-CPK066-G and 61-67-CPK067-G containing 50 base pairs of flanking homology to the *ADE1* open reading frame (ORF); the resultant amplicon was used to transform CEN.PK2-1C *erg*9Δ::*kan*^{r}_P_{MET3}-*ERG*9 and CEN.PK2-1D *erg*9Δ::*kan*^{r}_P_{MET3}-*ERG*9. *ade1* strains were selected for growth in the absence of leucine supplementation. The resultant clones were given the designation Y176 (MAT A) and Y177 (MAT alpha). Strain Y176 was transformed with *URA*3$^{-723\ to\ -224}$-*tHMG*-$P_{GAL1,10}$-*ERG*13-*URA*3$^{-223\ to\ 701}$ (released from pAM491 following digestion with PmeI) and *HIS*3$^{-32\ to\ -1000}$-*HIS*-*ERG*12-$P_{GAL1,10}$-*ERG*10-*HIS*3$^{504\text{-}1103}$ (released from pAM491 following digestion with PmeI).

Media and Growth Conditions. *Fermentation methods. Fermentation media.* The media used for this work were based on media described by van Hoek, et. al. (13). Vitamin solution contained biotin (0.05 g/L), calcium pantothenate (1 g/L), nicotinic acid (1 g/L), *myo*-inositol (25 g/L), thiamine HCl (1 g/L), pyridoxal HCl (1 g/L), and *p*-aminobenzoic acid (0.2 g/L). Trace metal solution contained $ZnSO_4{}^*7H_2O$ (5.75 g/L), $MnCl_2{}^*4H_2O$ (0.32 g/L), Anhydrous $CuSO_4$ (0.32 g/L), $CoCl_2{}^*6H_2O$ (0.47 g/L), $Na_2MoO_4{}^*2H_2O$ (0.48 g/L), $CaCl_2{}^*2H_2O$ (2.9 g/L), $FeSO_4{}^*7H_2O$ (2.8 g/L), 0.5 M EDTA (80 mL/L). Batch medium contained glucose (19.5 g/L), methionine (0.25 g/L, added at time of induction), $(NH_4)_2SO_4$ (15 g/L), KH_2PO_4 (8 g/L), $MgSO_4{}^*7H_2O$ (6.15 g/L), vitamin solution (12 mL/L), trace metals solution (10 mL/L). For the S288C-based strain Y87, the batch medium also contained 5 g/L lysine.

The bioreactor feed media varied for the different fermentation processes and the different strains. The glucose-fed-batch process used bioreactor feed base which contained 578 g/L glucose, 9 g/L KH_2PO_4, 5.12 g/L $MgSO_4{}^*7H_2O$, 3.5 g/L K_2SO_4, and 0.28 g/L Na_2SO_4. The glucose-fed phosphate-restricted fermentations used feed based identical to the glucose-fed-batch process, except that the feed base contained no KH_2PO_4.

All mixed glucose/ethanol processes used bioreactor feed base which contained 425 g/L glucose, 9 g/L KH_2PO_4, 5.12 g/L $MgSO_4{}^*7H_2O$, 3.5 g/L K_2SO_4, 0.28 g/L Na_2SO_4, 237 mL/L ethanol (95% vol/vol), and 1 g/L methionine added at induction.

For the glucose-fed-batch process, the glucose phosphate-restricted process, and the glucose and ethanol mixed feed process, two different feed media were prepared for the fermentation: preinduction feed medium, and induction feed medium (includes inducers). For both feed media, stock solutions of vitamins and trace metals were added to the bioreactor feed base as follows: 12 mL vitamin solution/liter feed base, and 10 mL trace metals solution/liter feed base. The preinduction feed media contained no other additions.

To the induction feed medium, two different inducers/repressors were added to the medium dependent on the strain. For

galactose-inducible strain, concentrated solutions of galactose and methionine were added to the induction feed media to bring the final concentrations to 10 g/L galactose and 1 g/L methionine. For strains containing Δ*gal80* (Y293 and Y319), no galactose was added to the induction media. All additions to the medium were made in a sterile hood.

Shake-flask medium for fermentation seed cultures. Seed medium for the fermentation preculture for the glucose-fed-batch fermentations and the glucose and ethanol fed-batch fermentations was the batch fermentation medium modified with the addition of 100 mL/L succinate buffer (0.5 M, pH 5.0) and no addition of methionine. For the glucose-fed phosphate-restricted fermentations, the seed media contained only 1 g/L KH_2PO_4.

Fermentation procedures.

Glucose feed and ethanol mixed feed process.

Seed cultures for bioreactors were prepared by inoculating 1 mL of frozen cells in 20% (vol/vol) glycerol into a 250 mL flask containing 50 mL of seed medium. Seed cultures were subcultured after approximately 24 h of growth at 30 °C by transferring 0.5 mL of culture into additional 250 mL flasks containing 50 mL seed medium. These flasks were grown for an additional 24 h at 30 °C to an OD_{600} of 5–10. Two seed flasks were pooled and used to inoculate batch medium in a 2 L bioreactor at a 10% vol/vol inoculum.

Strain Y87 grew significantly slower in the seed cultures and the bioreactor seed train was modified. For strain Y87, 1 mL of frozen cells in 20% (vol/vol) glycerol was used to inoculate a 250 mL flask containing 50 mL of seed medium. Seed culture was subcultured after approximately 24 h of growth at 30 °C by transferring 1 mL of culture into additional 250 mL flasks containing 50 mL seed medium. These flasks were grown for an additional 48 h at 30 °C to an OD_{600} of 5–10.

Fermentations took place in 2 L Sartorius Biostat-B plus twins with gas-flow ratio controllers. The pH was controlled automatically with the addition of approximately 10 M NH_4OH and maintained at pH 5. Temperature was maintained at 30 °C and airflow was supplied at a rate of 1 L/min. Dissolved oxygen was maintained at 40% with an agitation cascade followed by oxygen enrichment. Biospumex antifoam 200 K was used to control foam.

Bioreactors were inoculated and allowed to grow until all of the substrate in the batch medium was depleted. During the initial batch phase of the process, glucose was converted to both biomass and ethanol. After the glucose was depleted, the cells begin to consume the ethanol that was produced. The batch phase of the process continued until both the glucose in the batch medium and the ethanol produced in the batch phase was depleted. This depletion was detected by an increase in dissolved oxygen and a subsequent decrease in stir rate (as the controller compensated for the increasing dissolved oxygen). When the stir rate decreased (below 75% of the maximum revolutions per minute recorded so far in the run), the start of the exponential feed profile was automatically triggered. The feed rate during the exponential phase was determined by the following equations:

$$F = V\mu_{\text{set}}S_B e^{\mu_{\text{set}}(t-t_o)} \text{ and}$$

$$V = V_0 + V_{\text{feed}}.$$

F is the substrate mass flow rate (g substrate/hour), V is the liquid volume in the fermentor at a given time (liter), S_B is the concentration of substrate in the batch medium (19.5 g/L), μ_{set} is the specific feed rate (0.087 h^{-1}), t is the batch age (hour), t_0 is the batch age when the feed was initiated (hour), V_0 is the initial volume in the fermentor (0.7 L), and V_{feed} is the amount of volume added to the fermentor at a given time (liter). After the exponential feed phase of the fermentation was initiated, the exponential feed continued until the ratio of F/V (substrate mass flow rate/liquid volume in the fermentor) reached a preset maximum. For the glucose-fed-batch process and the glucose-fed phosphate-restricted process, the exponential feed continued until the ratio of F/V reached a maximum of 10 g glucose/hour per liter fermentor volume (Fig. S3). After reaching the end of exponential phase, the ratio of F/V was maintained constant at 10 g glucose/hour per liter fermentor volume for the remainder of the process. However, because the volume (V) continued to increase as more feed was added to the fermentor, the substrate mass flow rate (F) continued to increase until the volume reached the maximum working volume of the fermentor (approximately three times the starting volume). For the rest of the process, the fermentor volume was held constant by removing cell broth continuously from the reactor and the substrate mass flow rate (F) was held constant. Broth was removed at the same volumetric rate as feed delivery, in the same manner as a chemostat, though the system did not attain steady state. A feed rate profile can be found in Fig. S3.

In the glucose and ethanol mixed feed process, the exponential feed continued until the ratio of F/V reached a maximum of 8.6 g glucose and ethanol/hour per liter fermentor volume (Fig. S4). After reaching the end of exponential phase, the ratio of F/V was reduced to 4.3 g glucose and ethanol/hour per liter fermentor volume and maintained constant for the remainder of the process. However, because the volume (V) continued to increase as more feed was added to the fermentor, the substrate mass flow rate (F) continued to increase for the remainder of the run.

For galactose-inducible strains, the process was induced with the addition of 10 g/L galactose and 0.25 g/L methionine to the bioreactor after the culture reached an OD_{600} value of approximately 50. At this time, the feed bottle containing preinduction feed medium was exchanged for a feed bottle containing induction feed medium.

For strains containing Δgal80 (Y293 and Y319), 0.25 g/L of methionine was added to the bioreactor at approximately the time of the start of the fed-batch phase of the fermentation (no galactose was added). At this time, the feed bottle containing preinduction feed medium was exchanged for a feed bottle containing induction feed medium.

For fermentations of amorphadiene-producing strains, 10% vol/vol of methyl oleate was also added upon induction to capture the amorphadiene.

Ethanol feed process.

Cell bank preparation.

Strain Y293 was grown overnight in seed medium at 30 °C and 200 rpm until it reached an $OD_{600} = 2.4$. At that time the flask culture was placed on ice for 10 min. Three parts culture and two parts ice-cold sterile 50% glycerol were combined. One milliliter portions were frozen at −80 °C in cryovials.

Media.

The media used for this work were derived from the process described by van Hoek et al. (13). Seed medium contained tap water (350 mL/L), 2× batch base (500 mL/L), vitamin solution (12 mL/L), trace metals solution (10 mL/L), succinate (0.5 M, pH 5.0; 100 mL/L), Glucose*H_2O (30 mL/L). The 2× batch base contained KH_2PO_4 (16 g/L), (NH4)2SO4 (30 g/L), and $MgSO_4$*$7H_2O$ (12.3 g/L). Vitamin and trace metals solutions are the same as used in fermentation media. Bioreactor batch medium is described above. The following solutions are also required: 715 g/L glucose monohydrate, 60 g/L (0.5 M) succinic acid (pH 5.05), and 190 proof ethanol. The glucose monohydrate solution was prepared by dissolving the sugar in water with heating. The solutions were allowed to cool then filter sterilized.

The succinic acid was dissolved in water with heating. After the solution cooled, the pH was adjusted up to 5.05 with NaOH, and the solution was sterilized by autoclaving (45 min at 121 °C). Solutions were stored at room temperature unless otherwise indicated. The vitamins and trace metals were prepared fresh every two weeks.

The processes were run in 2-L Sartorious Biostat-B bioreactors. The pH was controlled automatically at pH with the addition of approximately 10 M NH_4OH. Temperature was maintained at 30 °C and airflow was supplied at a rate of 1.0 L/ min. Dissolved oxygen was maintained at 40% with an agitation cascade followed by oxygen enrichment. Biospumex antifoam 200 K was used to control foam.

Ethanol pulse feed.
Bioreactor batch medium was prepared by combining 2× batch base (at one-half of total bioreactor starting volume) with tap water and autoclaving. Trace metals, vitamins, and sugars were prepared as concentrated stocks and filter sterilized. Batch medium was brought up to 90% of final volume by adding filter sterilized 10× feed base as post sterile additions. The 10× feed base contained KH_2PO_4 (90 g/L), $MgSO_4{}^*7H_2O$ (51.2 g/L), K_2SO_4 (35 g/L), and Na_2SO_4 (2.8 g/L). The remaining 10% of starting volume was from the seed culture.

Strain Y293 was inoculated into 1 L of batch medium with 20 g/L glucose and grew until batch glucose and ethanol were depleted. At that time the feeding algorithm was initiated and a pure 95% ethanol solution was fed to the reactor with ethanol being the sole carbon source (Fig. S5). Ten hours after glucose was depleted, 0.25 g/L methionine was added through the head plate and 200 mL of autoclaved methyl oleate was pumped into the vessel to retain amorphadiene in culture solution. Because strain Y293 is Δgal80 mutant, galactose was not necessary for induction. Concentrated feed components were combined and injected through a septum in the bioreactor head plate with a syringe once per day according to how much ethanol volume had been delivered since the previous addition of feed components. Concentrated feed components were 25 g/L methionine (40 mL/L ethanol fed), 10× feed base (100 mL/L ethanol fed), vitamin solution (12 mL/L ethanol fed), trace metals solution (10 mL/L ethanol fed).

The ethanol fed fermentation processes used off-gas mass spectrometer CO_2 measurements to control ethanol feed delivery. As the culture grew in the initial batch glucose phase, some of the excess glucose was converted into ethanol. The CO_2 evolution was monitored using an off-gas analyzer and the computer algorithm assigned a variable (Cmax) which tracked the maximum value of the CO_2 percent in the off-gas. While growing on glucose, CO_2 was evolved rapidly until the glucose was depleted from the medium. At that point, the CO_2 evolution rate dropped to below 50% of its maximum value and the computer algorithm reset Cmax to the CO_2 value after the drop. The off-gas analyzer continued to monitor the CO_2 evolution as the remaining ethanol was consumed, updating Cmax as CO_2 evolution increased. Upon depletion of the batch ethanol, the CO_2 evolution rate dropped a second time. The ethanol feed pump was activated when the CO_2 value fell below 75% of the current Cmax. Ethanol was pumped into the vessel for 5 min, which delivered approximately 10 g ethanol to the culture. Cmax was reset to the value of the percent CO_2 at the time the pump turned off. This algorithm iterated during the course of the fermentation (Fig. S5).

Ethanol delivery was tailored to the cell demand with this feed algorithm and overfeeding was prevented. Ethanol did not accumulate in the medium during the fermentation.

Restricted ethanol feed.
Bioreactor batch medium was prepared by sterilizing 2× batch base (at 50% of total bioreactor starting volume) with 10× feed component solution (at 29% of bioreactor starting volume). Trace metals, vitamins, and sugars were prepared as concentrated stocks and filter sterilized. Batch medium was brought up to approximately 90% of final volume by adding filter-sterilized components (see ethanol pulse feed above) as post sterile additions. The remaining 10% of starting volume was from the seed culture.

The bioreactor was inoculated with strain Y293 into 0.7 L of batch medium with 20 g/L glucose and allowed to grow until glucose depleted. Upon depletion of the batch glucose indicated by a sharp decrease in CO_2 evolution measured by an off-gas mass spectrometer, the culture was automatically fed a concentrated glucose solution using a substrate restricted preprogrammed exponential feed. The feed rate during the exponential phase of the processes was determined by the following equations:

$$F = V\mu_{\text{set}} S_B e^{\mu_{\text{set}}(t-t_o)} \text{ and}$$

$$V = V_0 + V_{\text{feed}}.$$

F is the substrate mass flow rate (gram/hour), V is the liquid volume in the fermentor at a given time (liter), S_B is the concentration of substrate in the batch media (20 g/L), μ_{set} is the specific feed rate (0.087 h^{-1}), t is the batch age (hour), t_0 is the batch age when the feed was initiated (hour), V_0 is the initial volume in the fermentor (0.7 L), and V_{feed} is the total volume of feed added to the fermentor at a given time (liter). The exponential feed continued until the culture reached an OD_{600} of 50.

After reaching a maximum feed rate of 7.1 g/h per liter where the OD_{600} had a value of 50, the feed bottle was switched to a 95% (vol/vol) ethanol solution and the feed rate was set to a constant volumetric rate of 2.5 g/h per liter for the remainder of the run.

Shake-flask methods. Shake-flask media. Seed medium is described above. Flask production medium contained 40 g/L glucose, 0.5 g/L galactose, and 1.7 mM methionine.

Shake-flask procedures. All strains were confirmed with diagnostic PCR to contain the expected integration constructs and, where appropriate, all integrations were verified by sequence analysis. Single isolates of each strain from agar plates were grown for 18–24 h in 20 mL flask seed medium. Cultures were then inoculated into fresh seed medium containing 0.25 μM $CuSO_4$ at an OD_{600} of 0.05 and grown for an additional 18–24 h to an OD_{600} of between 2 and 3 (measured using a ThermoScientific Genesys 10 Vis spectrophotometer). Six hundred microliters of this culture was added to 400 μL of 50% glycerol and stored in 1 mL aliquots (25% glycerol) at −80 °C.

Seed vials of −80 °C were thawed to room temperature and inoculated into 20 mL flask seed medium. Cultures were grown for 18–24 h at 30 °C with shaking at 200 rpm. The following day, the cultures were diluted to an OD_{600} of 0.05 in 20 mL flask seed medium and grown for approximately 18 h at 30 °C with shaking at 200 rpm.

Cultures from the second overnight acclimation were diluted to an OD_{600} of 0.05 in 250 mL unbaffled flasks containing 25 mL flask production medium. Flasks contained an additional 5 mL isopropyl myristate (IPM) where indicated. All cultures were inoculated in triplicate and incubated at 30 °C for 72 h with shaking at 200 rpm. Flasks were sampled periodically for growth (OD_{600}), viability, and product titers. Viability was measured using the LIVE/DEAD *Funga* Light yeast viability kit for flow cytometry (Invitrogen) and a Guava Technologies EasyCyte Plus flow cytometer.

Development of phosphate-restricted fed-batch fermentations. Fermentation parameters were as described above. The control pro-

cess contained 8 g/L KH_2PO_4 in the batch medium, and 9 g/L KH_2PO_4 in the feed. The concentrations of KH_2PO_4 in both the batch and feed media were varied. Growth (OD_{600}) and production of amorpha-4,11-diene were measured. The strain used was Y337.

Glucose-limited, phosphate-restricted, fed-batch fermentation results. Fig. S6 shows growth, and Fig. S7 shows amorpha-4,11-diene production of glucose-limited fed-batch fermentations with various concentrations of KH_2PO_4 in the batch and feed media. The curves labeled 8 g/L KH_2PO_4 batch, 9 g/L KH_2PO_4 feed are the same as those labeled Y337 (*gal 1/10/7*Δ) in Fig. 3 of the manuscript, and Restricted Glucose Feed (Y337) in Fig. 4.

Mixed glucose/ethanol feed, phosphate-restricted, fed-batch fermentation results. Fig. S8 shows growth and Fig. S9 shows amorpha-4,11-diene production of mixed glucose/ethanol feed, fed-batch fermentations with various concentrations of KH_2PO_4 in the batch and feed media. The curves labeled 8 g/L KH_2PO_4 batch, 9 g/L KH_2PO_4 feed are the same as those labeled EtOH/glucose mixed feed (Y337) in Fig. 4 *A* and *B*.

Substrate and artemisinin costs as of October, 2011. Bulk galactose is approximately 100-fold more expensive than glucose (http://www.alibaba.com/).

Glucose cost is approximately $0.40/Kg (http://www.alibaba.com/), $0.072/mol, $0.012/Cmol.

Ethanol cost is approximately $2.30/US gallon (http://futures.tradingcharts.com/marketquotes/index.php3?sectorname=cbot), $0.61/L, $0.77/Kg, $0.035/mol, $0.018/Cmol.

Estimates of artemisinin demand, supply, and cost can be found at http://a2s2.org/index.php?id=33.

1. Rose MD, Winston F, Hieter P (1990) *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor Laboratory Press, Plainview, NY).
2. Guldener U, Heck S, Fielder T, Beinhauer J, Hegemann JH (1996) A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res* 24:2519–2524.
3. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15:1541–1553.
4. Boeke JD, Trueheart J, Natsoulis G, Fink GR (1987) 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol* 154:164–175.
5. Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Plainview, NY).
6. Horton RM (1997) In vitro recombination and mutagenesis of DNA. SOEing together tailor-made genes. *Methods Mol Biol* 67:141–149.
7. Longtine MS, et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* 14:953–961.
8. Paradise EM, Kirby J, Chan R, Keasling JD (2008) Redirection of flux through the FPP branch-point in *Saccharomyces cerevisiae* by down-regulating squalene synthase. *Biotechnol Bioeng* 100:371–378.
9. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.
10. Ro DK, et al. (2008) Induction of multiple pleiotropic drug resistance genes in yeast engineered to produce an increased level of antimalarial drug precursor, artemisinic acid. *BMC Biotechnol* 8:83.
11. Huxley C, Green ED, Dunham I (1990) Rapid assessment of *S. cerevisiae* mating type by PCR. *Trends Genet* 6:236.
12. Ro DK, et al. (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440:940–943.
13. van Hoek P, de Hulster E, van Dijken JP, Pronk JT (2000) Fermentative capacity in high-cell-density fed-batch cultures of baker's yeast. *Biotechnol Bioeng* 68:517–523.

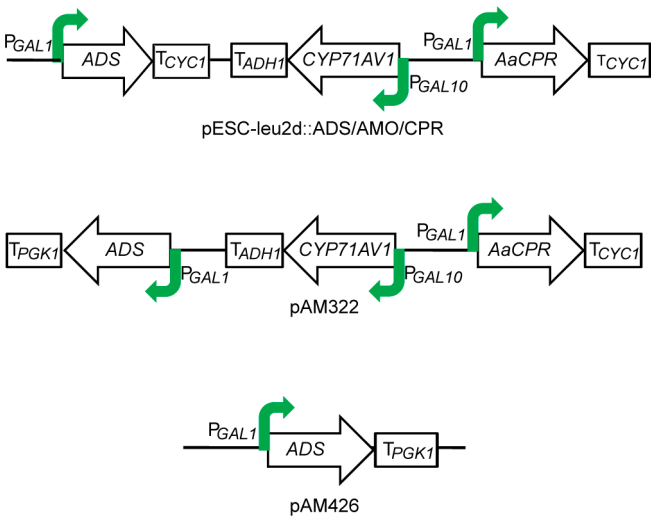

Fig. S1. Expression cassettes in 2 μ*leu2d* plasmids. pESC-Leu2d::ADS/AMO/CPR [where ADS is amorpha-4,11-diene synthase, AMO (amorphadiene oxidase) is CYP71AV1, and *Aa*CPR is *A. annua* cytochrome P450 reductase] is as described in ref. 10. pAM322 and pAM426 are described in the text.

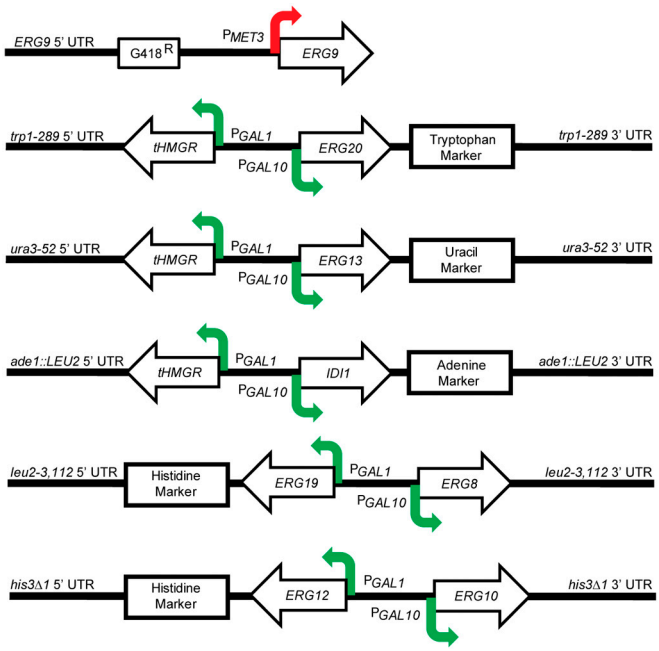

Fig. S2. Schematic of Gen. 2.0 genomic integrations.

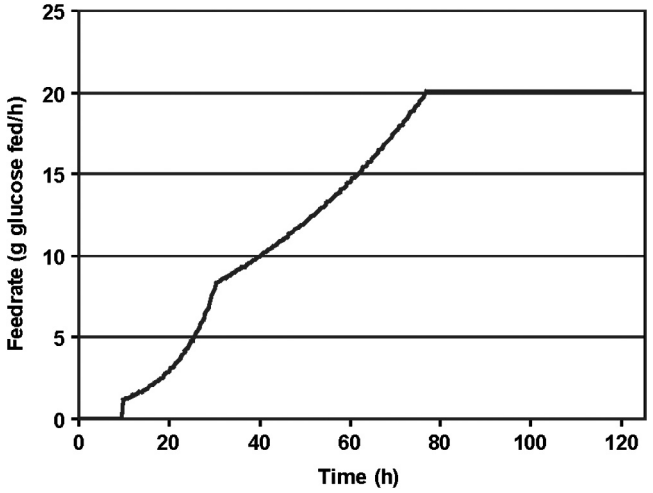

Fig. S3. Fed-batch fermentor feed profile for the glucose-fed-batch fermentation process and the glucose-fed phosphate-restricted fermentation process.

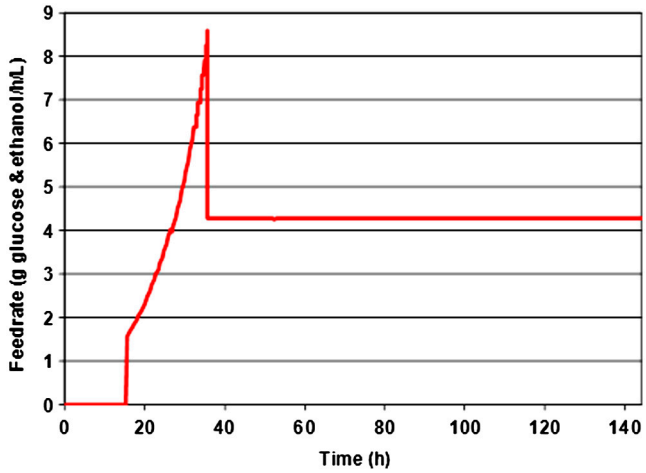

Fig. S4. Feed profile of glucose and ethanol mixed feed process. Feed rates are displayed as mass (gram) glucose and ethanol fed/hour per liter fermentor volume (F/V).

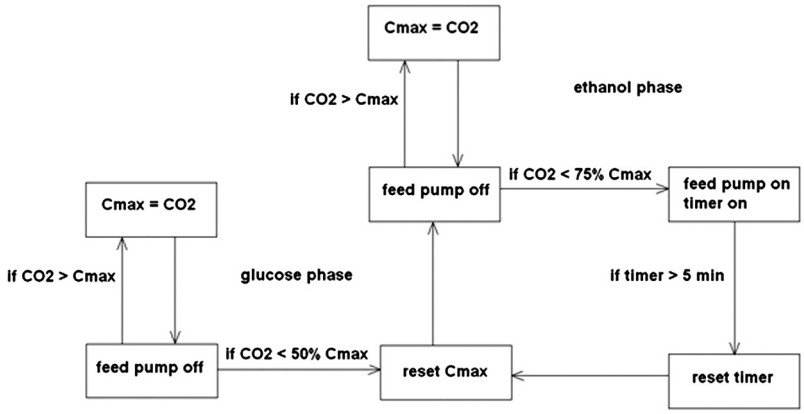

Fig. S5. Diagram of CO_2 control feed algorithm.

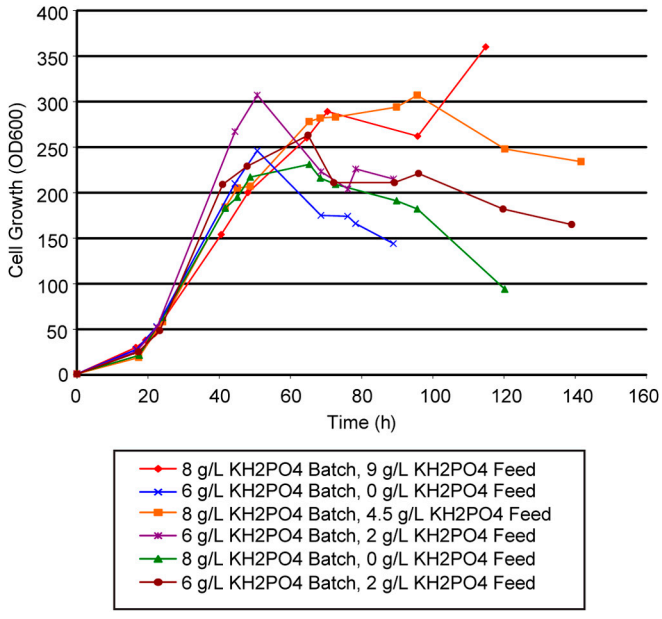

Fig. S6. Growth of Y337 in glucose-limited, phosphate-restricted, fed-batch fermentations.

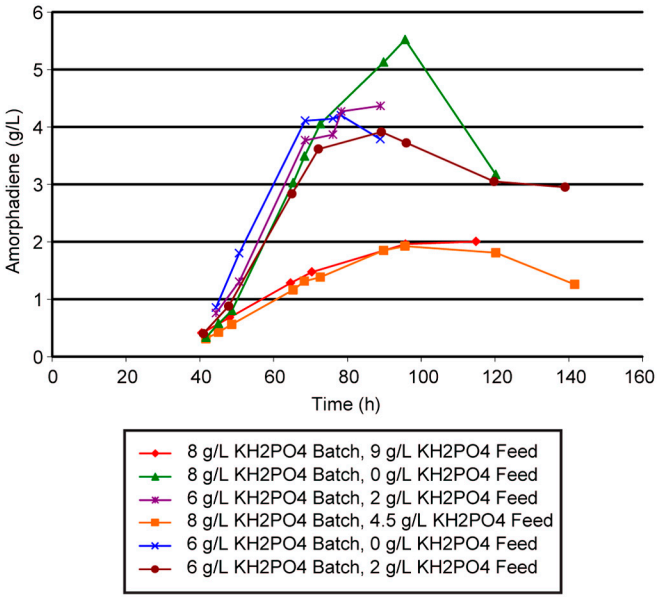

Fig. S7. Production of amorpha-4,11-diene by Y337 in glucose-limited, phosphate-restricted, fed-batch fermentations.

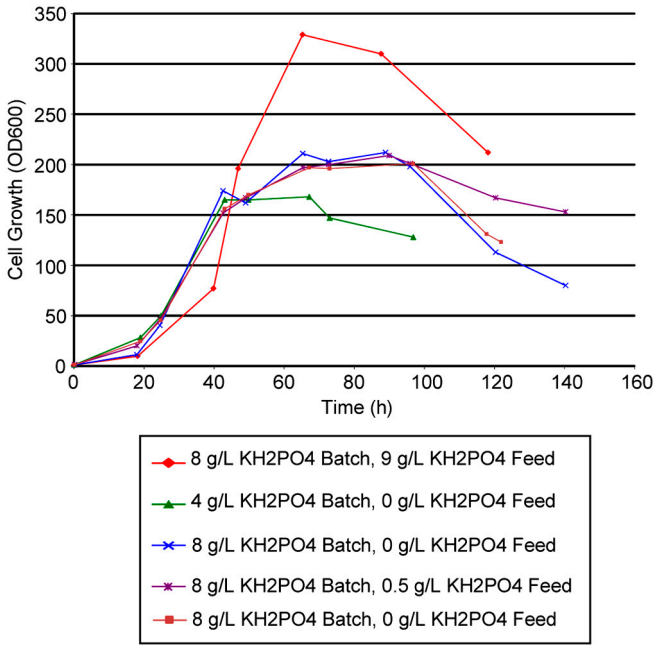

Fig. S8. Growth of Y337 in mixed glucose/ethanol feed, phosphate-restricted, fed-batch fermentations.

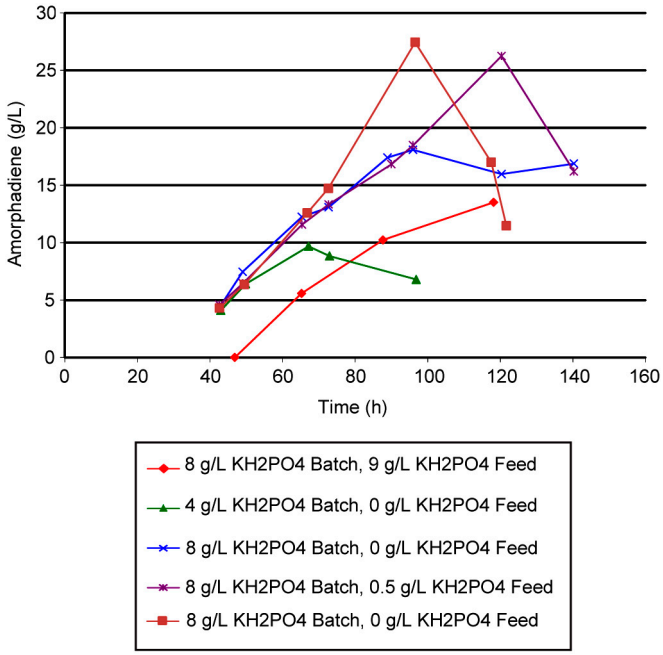

Fig. S9. Production of amorpha-4,11-diene by Y337 in mixed glucose/ethanol feed, phosphate-restricted, fed-batch fermentations.

Table S1. Strain genotypes and products, including precursor strains

Strain	Base strain	Genotype and [plasmid]	Source	Product
CEN.PK2-1C	—	*MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2*	EUROSCARF	—
CEN.PK2-1D	—	*MATα ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2*	EUROSCARF	—
Y176	**CEN.PK2**	*MATa ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2 erg9Δ::kan^r_P_{MET3}-ERG9 ade1Δ::CgLEU2*	This work	—
Y177	**CEN.PK2**	*MATα ura3-52 trp1-289 leu2-3,112 his3Δ1 MAL2-8C SUC2 erg9Δ::kan^r_P_{MET3}-ERG9 ade1Δ::CgLEU2*	This work	—
Gen 1.0				
EPY330	S288C	MATα his3Δ1 leu2Δ0 P_{GAL1}-*tHMG1*::*δ1* P_{GAL1}-*UPC2-1*::*δ2 erg9*::P_{MET3}-*ERG9*::*HIS3* P_{GAL1}-*ERG20*::*δ3* P_{GAL1}-*tHMG1*::*δ4* [pESC-leu2d-ADS/AMO/CPR]	(1)	AA
Y87	S288C	EPY330 *gal1Δ::K. lactis URA3 lys2Δ0*	This work	AA
Y108	CEN.PK2	*MATα erg9::Kan^r_P_{MET3}-ERG9 trp1::TRP1_P_{GAL1}-ERG20 his3::HIS_P_{GAL1}-tHMG1 leu2::LEU2_P_{GAL1}-tHMG1 ura3-52*	This work	—
Y135	CEN.PK2	*MATα erg9::Kan^r_P_{MET3}-ERG9 trp1::TRP1_P_{GAL1}-ERG20 his3::HIS_P_{GAL1}-tHMG1 leu2::HIS_P_{GAL1}-tHMG1 ura3::URA3_P_{GAL1}-upc2-1* [pAM322: 2μ-leu2d P_{GAL1}-*ADS* P_{GAL10}-*CYP71AV1* P_{GAL1}-*AaCPR* (codon opt)]	This work	AA
Y137	S288C	As Y87, but plasmid is pAM322	This work	AA
Y151	CEN.PK2	As Y135, but plasmid is pAM426; *2μ-leu2d P_{GAL1}-ADS (codon opt)*	This work	Amorph
Gen 2.0				
Y224	CEN.PK2	*MATa erg9Δ::kan^r_P_{MET3}-ERG9leu2–3,112::HIS_P_{GAL1}-MVD1_P_{GAL10}-ERG8 his3Δ1::HIS_P_{GAL1}-ERG12_P_{GAL10}-ERG10 ade1Δ::P_{GAL1}-tHMG1_P_{GAL10}-IDI1_ADE1 ura3-52::P_{GAL1}-tHMG1_P_{GAL10}_ERG13_URA3 trp1-289::P_{GAL1}-tHMG1_P_{GAL10}-ERG20_TRP1*[pAM322]	This work	AA
Y227	CEN.PK2	As Y224, but plasmid is pAM426	This work	Amorph
Y293	CEN.PK2	Y227 *gal80Δ::nat^r*	This work	Amorph
Y337	CEN.PK2	Y227 *gal1Δ gal7Δ gal10Δ::HphA*	This work	Amorph

Table S2. DNA sequence of oligonucleotide primers

Primer designation	Primer sequence 5′ → 3′
50-3-pw1-G	TGGATGGCGGCGTTAGTATC
50-3-pw2-G	ATGCAAAAAACATAAGACAAAGATCTGTTTAGCTTGCCTC
50-10-pw58-G	AAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACTATACACAGGAAACAGCTATGACC
50-10-pw59-G	GTTTTAAAGTTTGTGGATAATCGAAATCTCTTACATTGAAAACATTATCAGTTGTAAAACGACGGCCAGT
50-18-pw64-G	CAGGCAAGTGCACAAACAAT
50-18-pw65-G	CTAATTTCTTTTTCTGAAGCCATTTTGAGATCCGGGTTTT
50-18-pw66-G	AAAACCCGGATCTCAAAATGGCTTCAGAAAAAGAAATTAG
50-18-pw67-G	CATGGTCCTTATCTAGTTTG
50-18-pw71-G	CAATCAGTAAAAATCAACGGTTAACGACATTACTATATATATAATATAGGCATGGTCCTTATCTAGTTTG
50-27-pw80-G	CGATATCACAACTGTTACGA
50-56-pw100-G	GAGTGAACCTGCTGCCTGGCGTGCTCTGACTCAGTACATTTCATAGTGGATGGCGGCGTTAGTATC
50-56-pw101-G	CGTGTATACGTTTTCCGCTTCTGCTCTTCGTCTTTTCTCTTCTTCCGATATCACAACTGTTACGA
50-71-pw111-G	GAATTCGAGCTCGTTTAAAC
50-72-pw112-G	GTTTTCACCAATTGGTCTGCAGCCATTTTGAGATCCGGGTTTT
50-71-pw113-G	AAAACCCGGATCTCAAAATGGCTGCAGACCAATTGGTGAAAAC
50-71-pw114-G	TAGTTATGACAATTACAACA
50-100-pw162-G	CAAGTGATTAACGTCCACACAGGTATAGGGTTTCTGGACCATATGATACAGAATTCGAGCTCGTTTAAAC
50-100-pw163-G	TGGCTTCTCTTATGGCAACCGCAAGAGCCTTGAACGCACTCTCACTACGGTAGTTATGACAATTACAACA
50-100-pw166-G	ACATCGAGACCAAGAAGAACATTGCTGATGTGATGACAAAACCTCTTCCGTAGTTATGACAATTACAACA
50-104-pw176-G	CTTATTGGTTAGCTTTGATG
50-113-pw199-G	TCTAACTCCTTCCTTTTCGG
50-113-pw200-G	AAAGATGAATTGAAAAGCTT GCGGTAGTACGGATTAGAAG
50-113-pw201-G	CTTCTAATCCGTACTACCGCAAGCTTTTCAATTCATCTTT
61-13-pw242-G	CATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTTAATGTGGCTGTGGTTTCAGGGTCCATATCTAACTCCTTCCTTTTCGG
61-32-pw250-G	CAATAGGTGGTTAGCAATCGTCTTACTTTCTAACTTTTCTTACCTGAATTCGAGCTCGTTTAAAC
61-67-CPK001-G	GTTTAAACTACTATTAGCTGAATTGCCACT
61-67-CPK002-G	ACTGCAAAGTACACATATATCCCGGGTGTCAGCTCTTTTAGATCGG
61-67-CPK003-G	CCGATCTAAAAGAGCTGACACCCGGGATATATGTGTACTTTGCAGT
61-67-CPK004-G	GTTTAAACGGCGTCAGTCCACCAGCTAACA
61-67-CPK005-G	GTTTAAACTTGCTAAATTCGAGTGAAACAC
61-67-CPK006-G	AAAGATGAATTGAAAAGCTTCCCGGGTATGGACCCTGAAACCACAG
61-67-CPK007-G	CTGTGGTTTCAGGGTCCATACCCGGGAAGCTTTTCAATTCATCTTT
61-67-CPK008-G	GTTTAAACCCAACAATAATAATGTCAGATC
61-67-CPK009-G	GTTTAAACTACTCAGTATATTAAGTTTCGA
61-67-CPK010-G	ATCTCTCGCAAGAGTCAGACTGACTCCCGGGCGTGAATAAGCTTCGGGTGACCCTTATGGCATTCTTTTT
61-67-CPK011-G	AAAAAGAATGCCATAAGGGTCACCCGAAGCTTATTCACGCCCGGGAGTCAGTCTGACTCTTGCGAGAGAT
61-67-CPK012-G	GTTTAAACAATTTAGTGTCTGCGATGATGA
61-67-CPK013-G	GTTTAAACTATTGTGAGGGTCAGTTATTTC
61-67-CPK014alt-G	GCGGGGACGAGGCAAGCTAAACTTTAGTATATTCTTCGAAGAAA
61-67-CPK015alt-G	TTTCTTCGAAGAATATACTAAAGTTTAGCTTGCCTCGTCCCCGC
61-67-CPK017-G	CGATACTAACGCCGCCATCCACCCGGGAGAGGCTAGCAGAATTACCCTCCACGTTGATTG
61-67-CPK018-G	GTTTAAACGCCGCCGTTGTTGTTATTGTAG
61-67-CPK019-G	GTTTAAACTTTTCCAATAGGTGGTTAGCAA
61-67-CPK020-G	GGGTGACCCGGCGGGGACGAGGCAAGCTAAACGTCTTCCTTTCTCTTACCAAAGT
61-67-CPK021-G	ACTTTGGTAAGAGAAAGGAAGACGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCC
61-67-CPK022-G	AATATCATAAAAAAAAGAGAATCTTTCCCGGGTGGATGGCGGCGTTAGTATCGAATCGACAGC
61-67-CPK023-G	GCTGTCGATTCGATACTAACGCCGCCATCCACCCGGGAAAGATTCTCTTTTTTTATGATATT
61-67-CPK024-G	GTTTAAACGTGTTAACGTTTCTTTCGCCTACGTGGAAGGAGAATC
61-67-CPK025-G	TCCCCCCGGGTTAAAAAAAATCCTTGGACTAGTCA
61-67-CPK025-G	TCCCCCCGGGTTAAAAAAAATCCTTGGACTAGTCA
61-67-CPK026-G	AATCAATCAATCATCACATAAAATGGCTTCAGAAAAAGAAATTAGGA
61-67-CPK027-G	TCCTAATTTCTTTTTCTGAAGCCATTTTATGTGATGATTGATTGATT
61-67-CPK029-G	TTTTCACCAATTGGTCTGCAGCCATTTTATGTGATGATTGATTGATT
61-67-CPK030-G	AATCAATCAATCATCACATAAAATGGCTGCAGACCAATTGGTGAAAA
61-67-CPK031-G	TCCCCCCGGGAGTTATGACAATTACAACAACAGAA
61-67-CPK032-G	TCCCCCCGGGTATATATATATCATTGTTAT
61-67-CPK033-G	CAATCAATCAATCATCACATAAAATGAAACTCTCAACTAAACTTTGTTG
61-67-CPK034-G	CAACAAAGTTTAGTTGAGAGTTTCATTTTATGTGATGATTGATTGATTG
61-67-CPK035-G	TCCCCCCGGGAAAAGTAAGTCAAAAGGCAC
61-67-CPK035-G	TCCCCCCGGGAAAAGTAAGTCAAAAGGCAC
61-67-CPK036-G	TCAATCAATCAATCATCACATAAAATGTCTCAGAACGTTTACATTGTATCG
61-67-CPK037-G	CGATACAATGTAAACGTTCTGAGACATTTTATGTGATGATTGATTGATTGA
61-67-CPK038-G	TGCAGAAGTTAAGAACGGTAATGACATTTTATGTGATGATTGATTGATTGA
61-67-CPK039-G	TCAATCAATCAATCATCACATAAAATGTCATTACCGTTCTTAACTTCTGCA
61-67-CPK040-G	TCCCCCCGGGATGGTCTGCTTAAATTTCAT
61-67-CPK041-G	TCCCCCCGGGTAGCTTGTACCCATTAAAAGAATTTTATCATGCCG
61-67-CPK042-G	CAATCAATCAATCATCACATAAAATGTCAGAGTTGAGAGCCTTCAGTGCCC
61-67-CPK043-G	GGGCACTGAAGGCTCTCAACTCTGACATTTTATGTGATGATTGATTGATTG
61-67-CPK044-G	GGTAACGGATGCTGTGTAAACGGTCATTTTATGTGATGATTGATTGATTGA
61-67-CPK045-G	TCAATCAATCAATCATCACATAAAATGACCGTTTACACAGCATCCGTTACC
61-67-CPK046-G	TCCCCCCGGGTTTCTCATTCAAGTGGTAAC

Primer designation	Primer sequence 5′ → 3′
61-67-CPK047-G	TCCCCCCGGGTAAATAAAGAAAATAAAGTT
61-67-CPK048-G	CAATCAATCAATCATCACATAAAATGACTGCCGACAACAATAGTATGCCCC
61-67-CPK049-G	GGGGCATACTATTGTTGTCGGCAGTCATTTTATGTGATGATTGATTGATTG
61-67-CPK050-G	AATTTTTGAAAATTCAATATAAATGGCTTCAGAAAAAGAAATTAGGA
61-67-CPK051-G	TCCTAATTTCTTTTTCTGAAGCCATTTATATTGAATTTTCAAAAATT
61-67-CPK052-G	AGTTTTCACCAATTGGTCTGCAGCCATTATAGTTTTTTCTCCTTGACGTTA
61-67-CPK053-G	TAACGTCAAGGAGAAAAAACTATAATGGCTGCAGACCAATTGGTGAAAACT
61-67-CPK054-G	AATTTTTGAAAATTCAATATAAATGAAACTCTCAACTAAACTTTGTT
61-67-CPK055-G	AACAAAGTTTAGTTGAGAGTTTCATTTATATTGAATTTTCAAAAATT
61-67-CPK056-G	AATTTTTGAAAATTCAATATAAATGTCTCAGAACGTTTACATTGTAT
61-67-CPK057-G	ATACAATGTAAACGTTCTGAGACATTTATATTGAATTTTCAAAAATT
61-67-CPK058-G	TGCAGAAGTTAAGAACGGTAATGACATTATAGTTTTTTCTCCTTGACGTTA
61-67-CPK059-G	TAACGTCAAGGAGAAAAAACTATAATGTCATTACCGTTCTTAACTTCTGCA
61-67-CPK060-G	AATTTTTGAAAATTCAATATAAATGTCAGAGTTGAGAGCCTTCAGTG
61-67-CPK061-G	CACTGAAGGCTCTCAACTCTGACATTTATATTGAATTTTCAAAAATT
61-67-CPK062-G	GGTAACGGATGCTGTGTAAACGGTCATTATAGTTTTTTCTCCTTGACGTTA
61-67-CPK063-G	TAACGTCAAGGAGAAAAAACTATAATGACCGTTTACACAGCATCCGTTACC
61-67-CPK064-G	AATTTTTGAAAATTCAATATAAATGACTGCCGACAACAATAGTATGC
61-67-CPK066-G	GGTAAGACGGTTGGGTTTTATCTTTTGCAGTTGGTACTATTAAGAACAATCACAGGAAACAGCTATGACC
61-67-CPK067-G	TTGCGTTTTGTACTTTGGTTCGCTCAATTTTGCAGGTAGATAATCGAAAAGTTGTAAAACGACGGCCAGT
61-67-CPK065-G	GCATACTATTGTTGTCGGCAGTCATTTATATTGAATTTTCAAAAATT
61-67-CPK161-G	GACGCAGCGACTCGAGATCCATAGGAGCAACTCATGTCTGAACTTCAACGATTTCTATGA
61-67-CPK162-G	CAGACATGAGTTGCTCCTATGGATCTCGAGTCGCTGCGTCAACTTCAACGATTTCTATGA
91-014-CPK231-G	TTGTGATGCTAAAGTTATGAGTCTCGAGAAGTTAAGATTATATG
91-014-CPK232-G	CATATAATCTTAACTTCTCGAGACTCATAACTTTAGCATCACAA
91-014-CPK233-G	GTTTAAACTTCAAAGCTCGATGCCTCAT
91-014-CPK236-G	GTTTAAACGAGGCTCACCTAACAATTCA
91-014-CPK237-G	GATGTGTATTACTAGTGTCGACACTGCTGAAGAATTTGATTTTT
91-014-CPK238-G	AAAAATCAAATTCTTCAGCAGTGTCGACACTAGTAATACACATC