

SUPPORTING INFORMATION

3-Phosphoglycerate transhydrogenation instead of dehydrogenation alleviates redox state dependency of yeast *de novo* L-serine synthesis

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SUPPLEMENTAL REFERENCES

- [1] Kushnirov VV (2000). Rapid and reliable protein extraction from yeast. *Yeast* Jun 30, 16(9): 857-60
- [2] Becker-Kettern J, Paczia N, Conrotte JF, Kay DP, Guignard C, Jung PP, Linster CL. (2016). *Saccharomyces cerevisiae* Forms D-2-Hydroxyglutarate and Couples Its Degradation to D-Lactate Formation via a Cytosolic Transhydrogenase. *J Biol Chem* Mar 18;291(12):6036-58.

SUPPLEMENTAL METHODS

Detailed media compositions for yeast cultivations in this study.

Yeast cultivations were performed in chemically defined minimal media, chemically defined rich media (containing a sub selection of amino acids), synthetic complete media (containing all canonical amino acids), or complex media. All media were adjusted to an initial pH of 5.5 by addition of 1 M NaOH and sterilized by filtration. Solid media used for spotting assays were supplemented with 20 g/l of agar and sterilized by autoclaving.

Chemically defined minimal medium contained 1.7g/l YNB without ammonium sulfate, 5 g/l of either L-glutamate or ammonium sulfate, 80 mg/l L-methionine, 80 mg/l L-histidine, 240 mg/l L-leucine, and the indicated carbon source (added at a final concentration of 60 mM for glucose or 120 mM for pyruvate, glycerol, and lactate or 180 mM for acetate and ethanol)). Chemically defined rich medium contained in addition L-alanine, L-arginine, L-asparagine, L-aspartate, L-cysteine, L-glutamine, L-glutamate, L-glycine, L-isoleucine, L-lysine, L-proline, L-threonine, L-tryptophan, and L-valine (always 20 mg/l). If present, L-serine was added at a final concentration of 400 mg/l prior to sterilization, unless indicated otherwise. Synthetic complete medium contained 3.36 g/l SC-Glc-Glu-Pro-Ser+YNB powder (Sunrise Science Products; see ‘Bioreactor cultivations’ for detailed composition), 5 g/l of ammonium sulfate and 110 mM glucose. Complex medium (YPD) contained 10 g/l yeast extract, 20 g/l peptone, and 110 mM glucose.

For plasmid maintenance, uracil was always omitted from the media for growth and metabolic phenotyping of rescue strains (and the corresponding empty plasmid control strain), apart for the bioreactor cultivations, where serine was omitted from the medium to ensure plasmid maintenance.

Bioreactor cultivations were performed in synthetic complete medium prepared from a premixed powder (SC-Glc-Glu-Pro-Ser+YNB) from SunriseScience, supplemented with the indicated carbon source. One liter of this medium contained (in mg): biotin (0.002), boric acid (0.5), calcium chloride dehydrate (100), copper (II) sulfate pentahydrate (0.04), folic acid (0.002), inositol (87.6), iron (III) chloride (0.2), magnesium sulfate anhydrous (500), manganese sulfate monohydrate (0.4), niacin (0.4), 4-aminobenzoic acid (8.8), D-pantothenic acid hemicalcium salt (0.4), potassium iodide (0.1), potassium phosphate monobasic anhydrous (1000), pyridoxine hydrochloride (0.4), riboflavin (0.2), sodium chloride (100), sodium molybdate (0.2), thiamine hydrochloride (0.4), zinc sulfate monohydrate (0.4), adenine hemisulfate (21), L-alanine (85.6), L-arginine (85.6), L-asparagine monohydrate (85.6), L-aspartic acid (85.6), L-cysteine hydrochloride monohydrate (85.6), L-glycine (85.6), L-histidine hydrochloride monohydrate (85.6), L-isoleucine (85.6), L-leucine (173.4), L-lysine hydrochloride (85.6), L-methionine (85.6), L-phenylalanine (85.6), L-threonine (85.6), L-tryptophan (85.6), L-tyrosine (85.6), L-valine (85.6), and uracil (85.6).

Yeast cultivation experiments.

Spotting assays with serial dilutions were performed on agar plates containing complex medium, or chemically defined minimal medium supplemented with ammonium sulfate as a nitrogen source. Pre-cultures (5 ml) of all strains were grown in 50 ml shaking flasks in chemically defined minimal medium containing ammonium sulfate and shaken at 200 rpm at 30 °C for 18 h. After incubation, the pre-cultures were diluted to an optical density (OD₆₀₀) of 3, 2, and 1 using sterile water. The diluted cell suspensions were further diluted logarithmically in five steps (1:10, 1:100, 1:1,000, 1:10,000, 1:100,000) using a liquid handling robot (Integra Viaflow 384). 3 µl of each strain and each final dilution were spotted in triplicates on agar plates and left to dry before further incubation at 30 °C for 72 h, at which time point pictures were taken.

Microtiter plate cultivations for comparative metabolome analysis were performed in chemically defined minimal media containing L-glutamate as nitrogen source. Main cultures were inoculated from pre-cultures grown on serine free media of the same type. Pre-cultures (5 ml in 50 ml shaking flasks) of all strains were inoculated with 0.1% (v/v) of glycerol stock and incubated at 200 rpm and 30 °C for 18 h. The cell density of this pre-culture was then determined using a Multisizer Z3 equipped with a 30- μ m measurement capillary (Beckman Coulter). The main culture was adjusted to a starting cell density of 10^6 cells/ml and added to a 48-well plate (1 ml/well). The latter was covered with a sterile lid and incubated with a shaking frequency of 141.9 rpm at 30 °C for 80 h in a Tecan M200 Pro plate reader. Each strain was cultivated in quadruplicate. Due to the serine auxotrophy of the *ser3 Δ ser33 Δ* double mutant, analyses for this strain were done from main cultures that were directly inoculated with 1% (v/v) of glycerol stock. Culture aliquots for metabolite analysis as well as cell concentration and biovolume determination were taken at the entrance of stationary phase (according to the online OD signal).

Microtiter plate cultivations for growth phenotyping were performed in chemically defined minimal media and chemically defined rich media containing ammonium sulfate as nitrogen source after inoculation with 2% (v/v) of glycerol stock. The cultivations were then performed as described for the comparative metabolome analysis above.

Bioreactor cultivations were performed in synthetic complete medium containing ammonium sulfate as nitrogen source and 110 mM of glucose. The cultivations were carried out in 1 liter benchtop bioreactors (Bioblock, Eppendorf), with a working volume of 300 ml in chemostat mode at a constant air flow of 0.5 vvm, a constant agitation rate of 300 rpm, and a constant temperature of 30 °C. While the inflow of fresh medium was changed within one experiment every three residence times from 15 ml/h (resulting in an dilution rate of 0.05 h^{-1} and a residence time of 20 h), to 30 ml/h (resulting in a dilution rate of 0.1 h^{-1} and a residence time of 10 h), to 45 ml/h (resulting

in a dilution rate of 0.15 h^{-1} and a residence time of 6.33 h), to 60 ml/h (resulting in a dilution rate of 0.2 h^{-1} and a residence time of 5 h), the outflow was kept constant at a flow rate of 150 ml/h from a tube adjusted to the surface level of the working volume at 300 rpm.

Additional growth phenotyping in shaking flasks was performed in chemically defined minimal medium containing ammonium sulfate as the nitrogen source. Shaking flask cultivations were performed at $30\text{ }^{\circ}\text{C}$ and 200 rpm in 50 ml flasks with a working volume of 7.5 ml inoculated with 0.1% (v/v) of glycerol stock. Growth was measured by determining the cell concentration after 96 h using a Beckman Multisizer Z3 (Beckman Coulter).

Analysis of Ser3, Ser33, and human PHGDH expression in yeast rescue strains using Western blot and quantitative RT-PCR.

Sample preparation. BY4741 wild-type, FY4 wild-type, and *ser3 Δ ser33 Δ* mutant (transformed with empty vector) and the *SER3*, *SER33*, or h*PHGDH* rescue strains (BY4741 *ser3 Δ ser33 Δ* background) were grown in 250 ml flasks with a working volume of 10 ml inoculated at a OD_{600} of 0.05 using chemically defined minimal medium as described above containing 60 mM glucose, 240 mg/l L-serine, and 80 mg/l uracil (the latter for the BY4741 and FY4 control strains only). After 17 h of cultivation at $30\text{ }^{\circ}\text{C}$ and 200 rpm, 2 ml of culture were centrifuged for 5 min at $4500\times g$ and ambient temperature and the supernatant discarded. The cell pellets were stored at $-80\text{ }^{\circ}\text{C}$ until further analysis.

Western blot. Yeast proteins were extracted from the cell pellets for SDS-PAGE and Western blot analysis as previously described [1]. Cell pellets were thawed on ice, resuspended in 200 μl 0.1 M NaOH, and incubated for 5 min at room temperature. After centrifugation for 5 min at $15000\times g$ and at room temperature, cells were resuspended in 200 μl Laemmli buffer, boiled for 3 min at

95 °C and again centrifuged. The supernatant was used directly to separate soluble proteins on a Mini-PROTEAN® TGX™ Precast Gel (10% polyacrylamide, 15 wells; BioRad). Recombinant purified his₆-tagged Ser3 (1.09 µg), Ser33 (2.46 µg), and *h*PHGDH (0.04 µg) were also loaded for comparison. Proteins were either stained using the PageBlue™ Protein Staining Solution (Thermo Fisher Scientific) or transferred to a PVDF membrane (iBlot™ 2 Transfer Stacks, PVDF, mini; Thermo Fisher Scientific). After transfer, the membrane was blocked overnight at 4 °C in 5% BSA (Albumin Fraction V, pH 7.0, blotting grade; AppliChem) dissolved in T-TBS (containing 0.1 % Tween 20; Sigma). The membrane was incubated with the primary antibody targeted against human PHGDH (1:750 in T-TBS; #HPA021241 from Sigma) for 4 h at room temperature, followed by 3 washes with T-TBS. The membrane was then incubated with a secondary antibody coupled with a horse-radish peroxidase (Anti-rabbit IgG, 1:5000 in T-TBS, Cell Signaling Technology, # 7074S) for 2 hours at room temperature and washed 5 times for at least 5 minutes. Proteins were detected using the ECL™ Prime Western Blotting Detection Reagent (GE Healthcare) according to the manufacturer's instructions and exposing the membrane for 2 minutes in an Odyssey® Fc Imaging system (Westburg) using the chemiluminescence detection channel.

Quantitative RT-PCR. Cell pellets were resuspended in 600 µl RLT buffer and RNA extracted using the RNeasy Mini Kit (Qiagen) as instructed by the supplier combined with mechanical cell disruption (2 x 20 sec, with 30 sec pause in a Precellys, Bertin). RNA was eluted with RNase- and DNase-free water in a final volume of 40 µl. Contaminating DNA was removed using the TURBO™ DNase kit (Invitrogen) by incubating 5.5-9.6 µg of total RNA with 1.5 Units of DNase in a final volume of 30 µl following the manufacturer's instructions. Reverse transcription was performed with the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) according to the manufacturer's instructions using Random Hexamer primers and 500

ng of total RNA as template. The qRT-PCR analysis was performed in LightCycler480 Multiwell plates (384-well plate; Roche) using the iQ SYBR Green Supermix (BioRad) according to manufacturer's instructions (10 μ l total reaction volume including 0.3 μ M of both forward and reverse primers and a final 1:100 dilution of cDNA). -Fold changes were calculated using the $2^{-\Delta\Delta C_t}$ -method with *ALG9* and *TFCI* as reference genes. The sequences of the qRT-PCR primers used are given in Table S3.

SUPPLEMENTAL TABLES

Supplemental Table S1

Yeast/bacterial strains and plasmids used in this study. The wild-type strains used were the *Saccharomyces cerevisiae* BY4741 strain (*MATa*; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*), the prototrophic FY4 strain (*MATa*), the *Escherichia coli* DH5α strain (*fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*), and the *Escherichia coli* BL21(DE3) strain (*F⁻ ompT gal dcm lon hsdSB(rB⁻ mB⁻) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*). Plasmids for protein expression in yeast (pAG416-GPD, Addgene plasmid #14148) or bacteria (pDONR221 and pDEST-527) were generated using the Gateway technology. *Ec*, *E. coli*; GPD, glyceraldehyde-3-phosphate dehydrogenase; ORF, open reading frame; *Sce*, *S. cerevisiae*.

<i>Strains</i>		
<i>Name</i>	<i>Organism & strain</i>	<i>Genetic modification</i>
<i>ser3Δser33Δ</i>	<i>Sce</i> BY4741	<i>ser3::KanMX</i> ; <i>ser33::KanMX</i>
<i>ser3Δser33Δ</i> + control	<i>Sce</i> BY4741	<i>ser3::KanMX</i> ; <i>ser33::KanMX</i> ; pAG416-GPD- <i>ccdB</i>
<i>ser3Δser33Δ</i> + <i>SER3</i>	<i>Sce</i> BY4741	<i>ser3::KanMX</i> ; <i>ser33::KanMX</i> ; pAG416-GPD- <i>SER3</i>
<i>ser3Δser33Δ</i> + <i>SER33</i>	<i>Sce</i> BY4741	<i>ser3::KanMX</i> ; <i>ser33::KanMX</i> ; pAG416-GPD- <i>SER33</i>
<i>ser3Δser33Δ</i> + h <i>PHGDH</i>	<i>Sce</i> BY4741	<i>ser3::KanMX</i> ; <i>ser33::KanMX</i> ; pAG416-GPD-h <i>PHGDH</i>
DH5α pDONR221- <i>SER3</i>	<i>Ec</i> DH5α	pDONR221- <i>SER3</i>
DH5α pDONR221- <i>SER33</i>	<i>Ec</i> DH5α	pDONR221- <i>SER33</i>
DH5α pDONR221-h <i>PHGDH</i>	<i>Ec</i> DH5α	pDONR221-h <i>PHGDH</i>
DH5α pDest-527-his ₆ - <i>SER3</i>	<i>Ec</i> DH5α	pDest-527-his ₆ - <i>SER3</i>
DH5α pDest-527-his ₆ - <i>SER33</i>	<i>Ec</i> DH5α	pDest-527-his ₆ - <i>SER33</i>
DH5α pET28a(+)-h <i>PHGDH</i>	<i>Ec</i> DH5α	pET28a(+)-h <i>PHGDH</i>
DH5α pET15b- <i>PSAT1</i>	<i>Ec</i> DH5α	pET15b- <i>PSAT1</i>
BL21(DE3) pDest-527-his ₆ - <i>SER3</i>	<i>Ec</i> BL21(DE3)	pDest-527-his ₆ - <i>SER3</i>
BL21(DE3) pDest-527-his ₆ - <i>SER33</i>	<i>Ec</i> BL21(DE3)	pDest-527-his ₆ - <i>SER33</i>
BL21(DE3) pET28a(+)-h <i>PHGDH</i>	<i>Ec</i> BL21(DE3)	pET28a(+)-h <i>PHGDH</i>
BL21(DE3) pET15b- <i>PSAT1</i>	<i>Ec</i> BL21(DE3)	pET15b- <i>PSAT1</i>

Plasmids	
pDONR221	Gateway Entry vector for attB-flanked ORFs
pDONR221- <i>SER3</i>	Gateway Entry clone containing the <i>SER3</i> ORF
pDONR221- <i>SER33</i>	Gateway Entry clone containing the <i>SER33</i> ORF
pDONR221-h <i>PHGDH</i>	Gateway Entry clone containing the human <i>PHGDH</i> ORF
pDest-527	Gateway Destination vector for bacterial expression with N-terminal
pDest-527-his ₆ - <i>SER3</i>	Gateway Expression clone for bacterial expression of his ₆ - <i>SER3</i>
pDest-527-his ₆ - <i>SER33</i>	Gateway Expression clone for bacterial expression of his ₆ - <i>SER33</i>
pAG416-GPD-ccdB	Gateway Destination vector for protein expression in yeast
pAG416-GPD- <i>SER3</i>	Gateway Expression clone for expression of <i>SER3</i> in yeast
pAG416-GPD- <i>SER33</i>	Gateway Expression clone for expression of <i>SER33</i> in yeast
pAG416-GPD-h <i>PHGDH</i>	Gateway Expression clone for expression of h <i>PHGDH</i> in yeast

Supplemental Table S2

Overview of yeast cultivation experiments in this study. Data shown in main or supplemental figures was produced by cultivation of the indicated strains in the indicated cultivation device in the indicated media. ALA, L-Alanine; ARG, L-Arginine; ASN, L-Asparagine; ASP, L-Aspartate; CYS, L-Cysteine; GLN, L-Glutamine; GLU, L-Glutamate; GLY, L-Glycine; HIS, L-Histidine; ILE, L-Isoleucine; LYS, L-Lysine; MET, L-Methionine; PRO, L-Proline; THR, L-Threonine; TRP, L-Tryptophan; VAL, L-Valine; SER, L-Serine; LEU, L-Leucine; ‘+’ indicates ‘supplementation’; ‘-’ indicates ‘no supplementation’. Detailed descriptions of each experiment can be found in the Supplemental Methods section.

Experiment/ figure	Strains	Cultivation device	Media		
			C- source	N-source	Supplementation
2-HG analysis Figure 7	<i>ser3Δ ser33Δ</i> + ctrl	<i>Microtiter plate (48- well plate)</i>	Glucose	GLU	Chemically defined minimal
	<i>ser3Δ ser33Δ</i> + <i>SER3</i>				
	<i>ser3Δ ser33Δ</i> + <i>SER33</i>				+MET +HIS + LEU
	<i>ser3Δ ser33Δ</i> + h <i>PHGDH</i>				-SER or +SER

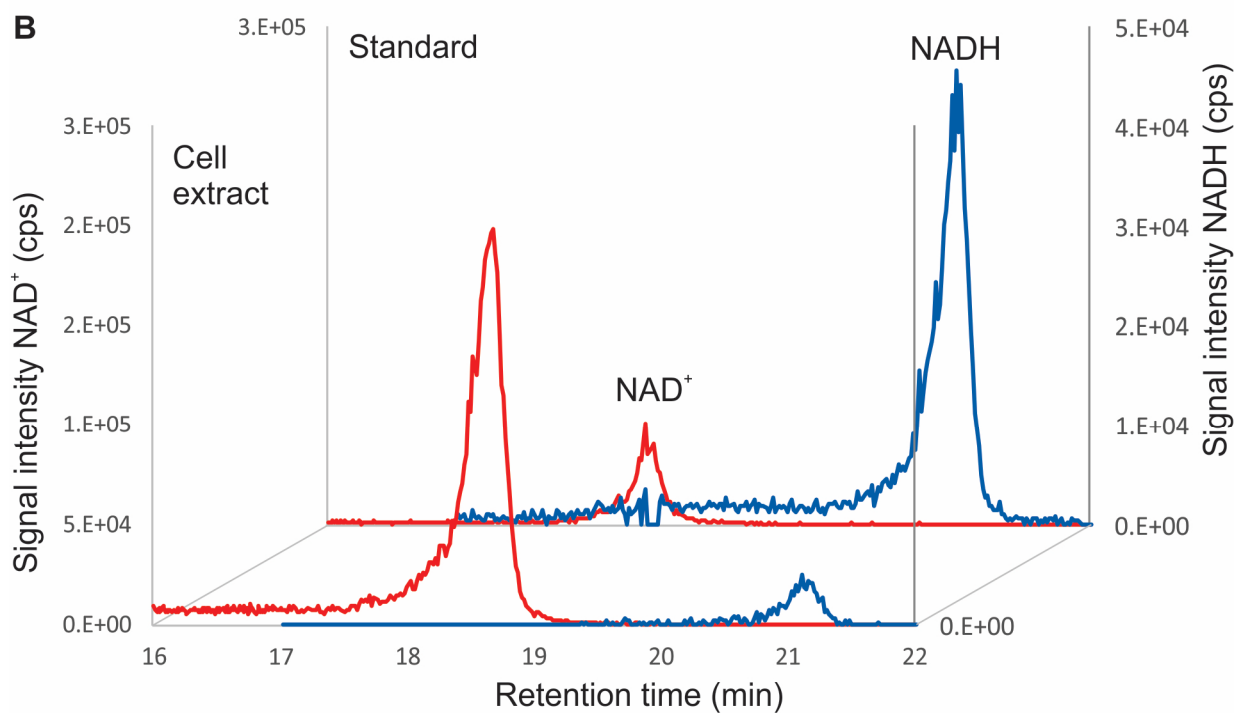
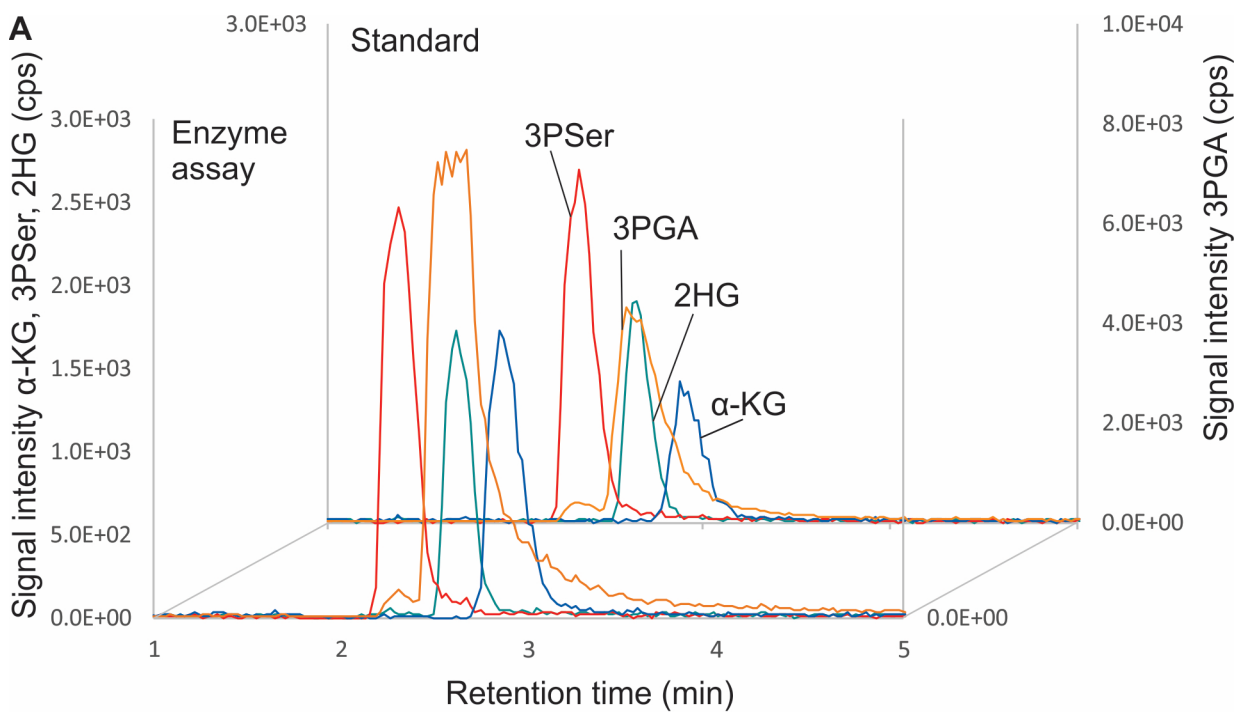
<i>NAD⁺/NADH analysis</i> Figure 8	<i>Sce</i> BY4741 <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Bioreactor in chemostat mode</i>	Glucose	Ammonium sulfate	Synthetic complete (all amino acids, except the below mentioned) - SER - GLU - GLN - PRO
<i>Spotting assay</i> Figure 9A	<i>ser3Δ ser33Δ</i> + ctrl <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Agar plates, Static incubation</i>	Glucose or Pyruvate	Ammonium sulfate	Chemically defined minimal -SER or +SER
<i>Growth phenotyping</i> Figure 9B	<i>ser3Δ ser33Δ</i> + ctrl <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Microtiter plate (48-well plate)</i>	Glucose or Pyruvate	Ammonium sulfate	Chemically defined minimal -SER or +SER Chemically defined rich +ALA +ARG +ASN +ASP +CYS +GLN +GLU +GLY +HIS +ILE +LYS +MET +PRO +THR +TRP +VAL -SER or +SER
<i>NAD⁺/NADH analysis</i> Figure 9C	BY4741	<i>Shaking flask</i>	Glucose or Pyruvate	Ammonium sulfate	Chemically defined minimal -SER
<i>Expression analysis</i> Supplemental Figure S4	FY4 BY4741 <i>ser3Δ ser33Δ</i> + ctrl <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Shaking flask</i>	Glucose	Ammonium sulfate	Chemically defined minimal +SER
<i>Spotting assay</i> Supplemental Figure S5	<i>ser3Δ ser33Δ</i> + ctrl <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Agar plates, Static incubation</i>	Glucose or Lactate or Glycerol Or Acetate or Ethanol	Ammonium sulfate	Complex medium Chemically defined minimal -SER or +SER
<i>Growth phenotyping</i> Supplemental Figure S6	<i>ser3Δ ser33Δ</i> + ctrl <i>ser3Δ ser33Δ</i> + <i>SER3</i> <i>ser3Δ ser33Δ</i> + <i>SER33</i> <i>ser3Δ ser33Δ</i> + hPHGDH	<i>Shaking flask</i>	Glucose or Acetate or Ethanol or Glycerol or Lactate or Pyruvate	Ammonium sulfate	Chemically defined minimal -SER or +SER

Supplemental Table S3

Primers for qRT-PCR used in this study.

<i>Gene</i>	<i>Forward primer</i>	<i>Reverse primer</i>
<i>ALG9</i>	5'-CCGCATTGCGACTGCTGTTTG-3'	5'-CAGGAGCAAGCTTCCCGTAA-3'
<i>TFC1</i>	5'-ATGTACCAAAGCCACCACCC-3'	5'-TGCAACATCAGGTACCACGG-3'
<i>SER3</i>	5'-CTGCTATGAAGGATGGCGCT-3'	5'-TTGGCTTTCACGGCTTGGAT-3'
<i>SER33</i>	5'-AAGTCGCTGCTAGGTGTTGG-3'	5'-AGGCCCATAGCTTCTGCAAG-3'
<i>PHGDH</i>	5'-GTCATTGTCGGCCTCCTGAA-3'	5'-AGCAGCTTAGCGTTCACCAA-3'

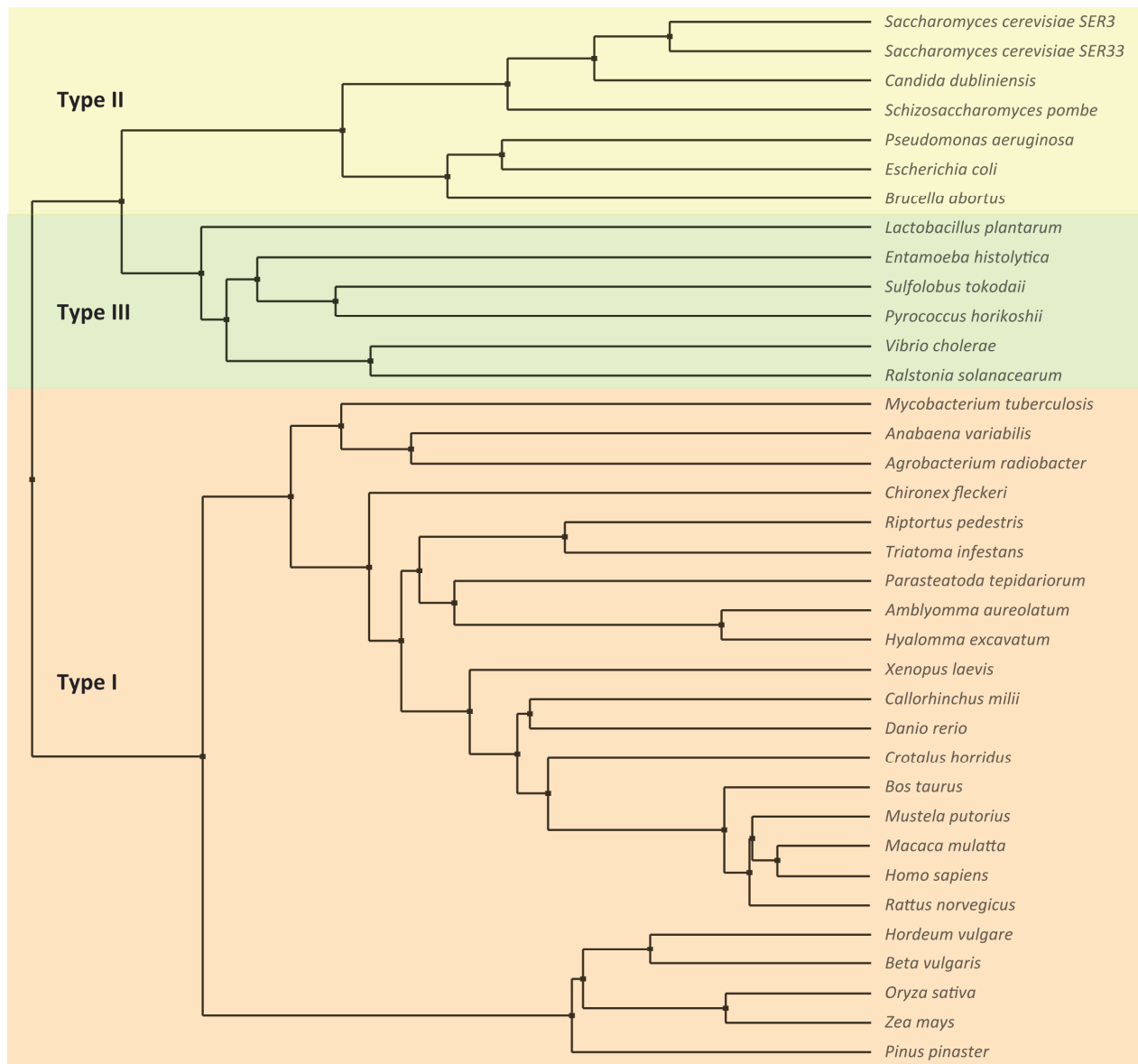
SUPPLEMENTAL FIGURES



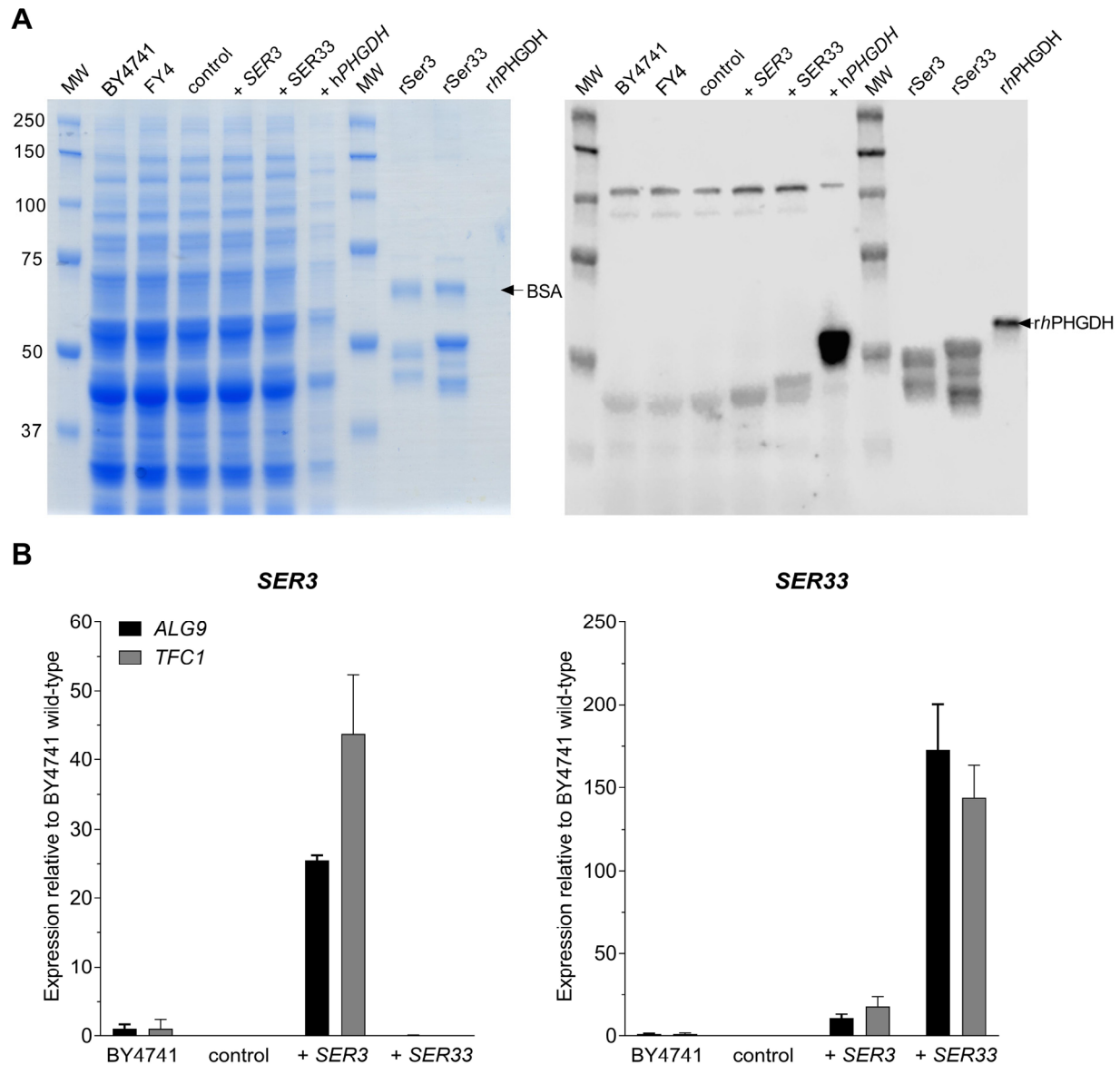
Suppl. Fig. S1: **Targeted LC-MS(/MS) detection of 3PGA, 3PSer, α -KG, and 2HG and of NAD(H) coenzyme in enzymatic assays and cell extracts.** (A) Representative extracted ion chromatograms of a chemically pure standard solution containing alpha-ketoglutarate (α -KG), 3-phosphoserine (3PSer), 2-hydroxyglutarate (2HG), and 3-phosphoglycerate (3PGA) and of a 3PGA- α KG transhydrogenase enzyme assay. Metabolite identification was based on retention time comparison of the signals in standard and sample, and on specific m/z transitions between precursor and fragment ion detected by tandem mass spectrometry (API400 LIT MS) (145.0 \rightarrow 56.9 for α -KG; 147.0 \rightarrow 84.9 for 2HG; 183.9 \rightarrow 79.1 for 3PSer; 185.0 \rightarrow 96.7 for 3PGA). (B) Representative extracted ion chromatograms of a chemically pure standard solution (back) containing NAD⁺ (red) and NADH (blue) and of a yeast cell extract (front). Metabolite identification was based on retention time comparison of the signals in standard and sample, and on the accurate mass detected by the high-resolution mass spectrometer (QExactive Orbitrap MS), with a mass error below 2 ppm based on the theoretical m/z of 662.101296 (NAD⁺) and 664.116946 (NADH).

Suppl. Fig S2: **Structure-weighted sequence alignment of Ser3 and Ser33 with other PHGDHs.** Alignment between representative PHGDH crystal structures and protein sequences from different sources. The top numbers indicate the overall alignment column numbering. The first row represents the conservation histogram of the overall alignment. The second row represents the sequence regions corresponding to the SBD (orange), NBD (yellow), ASB (green) and ACT (blue) domains. The third row shows the consensus sequence of the overall alignment. The next rows correspond to PHGDH amino acid sequences with single letter code residues in Clustal color. Sequences are shown in the following order: Type II PHGDHs from *S. cerevisiae* Ser3 (P40054, Uniprot code here and thereafter), *S. cerevisiae* Ser33 (P40510), *E. coli* (PDB: 1TBA; P0A9T0), *B. abortus* (PDB: 3K5P; Q2YK82), *S. pombe* (P87228), *Candida dubliniensis* (B9W9J) and *P. aeruginosa* (A0A0D6FHI1). Type III PHGDHs from *Ralstonia solanacearum* (PDB: 3EVT; Q8Y3G4), *Vibrio cholerae* (PDB: 4S1V; A0A0H2UKZ7), *Entamoeba histolytica* (PDB: 4NJO; Q76KF5), *Sulfolobus tokodaii* (PDB: 2EKL; Q972A9), *Pyrococcus horikoshii* (PDB: 1WWK; O50095) and *Lactobacillus plantarum* (PDB: 3EVT; F9URQ7). Type I PHGDHs from *M. tuberculosis* (PDB: 3DDN; P9WNX3), *Homo sapiens* (PDB: 2G76; O43175), *Pinus pinaster* (G3C901), *Zea mays* (B6SKK1), *Beta vulgaris* (A0A1Z4EAX4), *Oryza sativa* (Q6ZAA5), *Hordeum vulgare* (F2D807), *Macaca mulatta* (H9FST6), *Mustela putorius* (G9KGC8), *Rattus norvegicus* (O08651), *Bos taurus* (Q5EAD2), *Crotalus horridus* (T1DNB1), *Danio rerio* (Q6PHU9), *Callorhinchus milii* (V9KMU6), *Xenopus laevis* (A1L3N1), *Amblyomma aureolatum* (A0A1E1XAE4), *Hyalomma excavatum* (A0A131XJZ9), *Triatoma infestans* (A0A023F8D6), *Parasteatoda tepidariorum* (A0A2L2Y071), *Riptortus pedestris* (R4WJT3), *Chironex fleckeri* (D1FX73), *Agrobacterium radiobacter* (B9J9G2) and *Anabaena variabilis* (Q3M6M2). The secondary structure elements from the crystal structures are shown as painted yellow (α-helices) and green (β-strands) boxes. Empty boxes represent predicted secondary structure deduced from

homology modeling of Ser33 using *Ec*PHGDH and *Ba*PHGDH crystal structures as templates. Red empty boxes are areas not visible in the crystal structure. On top of the conservation histogram, residues implicated in substrate (empty triangles), NAD (solid triangles) or the regulator L-serine (asterisk) binding are indicated. In addition, residues participating in catalysis are marked with empty circles.

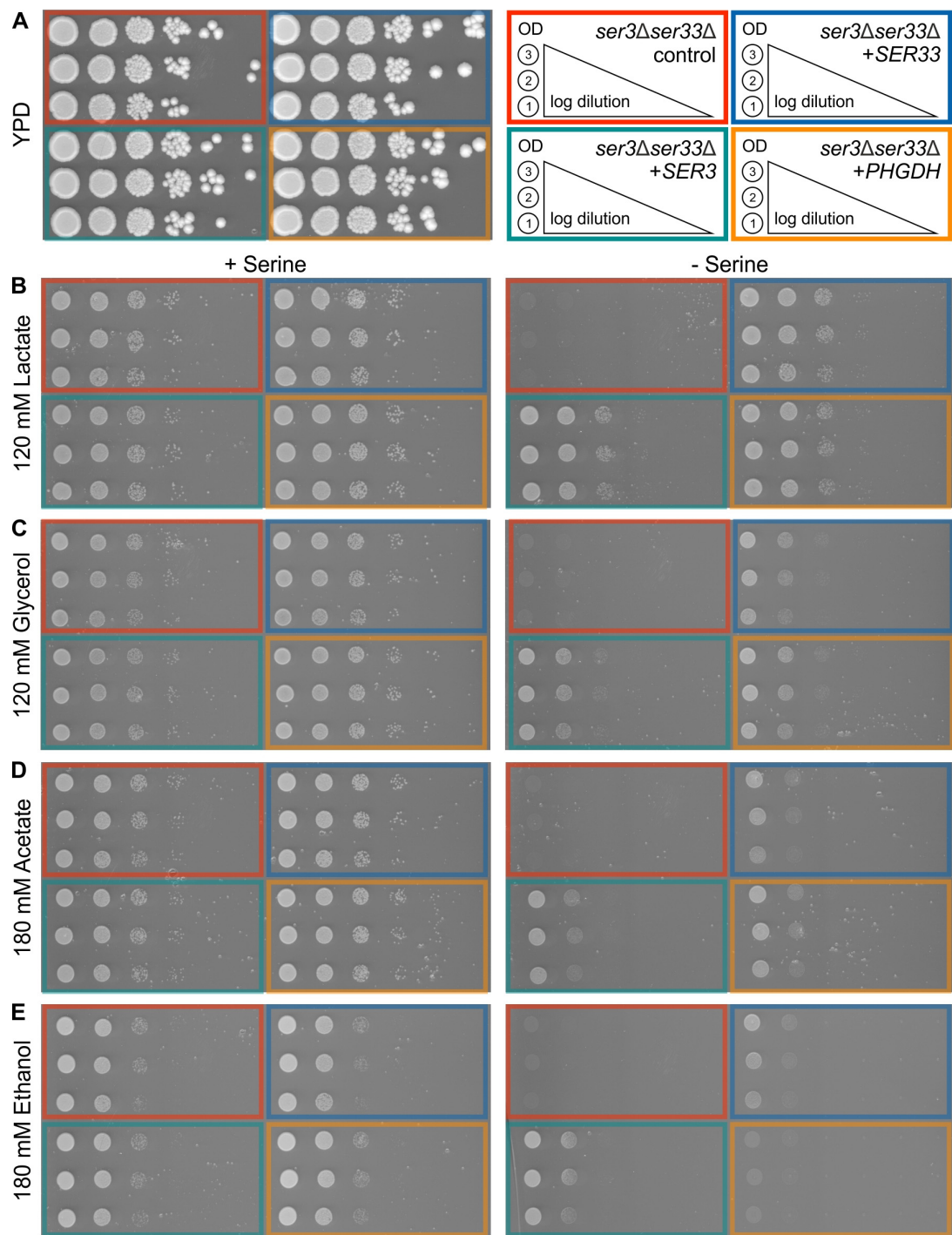


Suppl. Fig. S3: **Yeast Ser3 and Ser33 cluster with Type II PHGDHs.** The derived average distance Blosum62 phylogenetic tree from a structure-weighted alignment of Ser3 and Ser33 with other PHGDHs (See also Suppl. Fig. S2). Clusters indicating different architectures are shadowed for Type I (orange), Type II (yellow) and Type III (green). PHGDH sources are indicated for Type II, *S. cerevisiae* Ser3 (P40054), *S. cerevisiae* Ser33 (P40510), *E. coli* (PDB: 1YBA; P0A9T0), *B. abortus* (PDB: 3K5P; Q2YK82), *S. pombe* (P87228), *Candida dubliniensis* (B9W9J) and *P. aeruginosa* (A0A0D6FHI1). Type III PHGDHs from *Ralstonia solanacearum* (PDB: 3EVT; Q8Y3G4), *Vibrio cholerae* (PDB: 4S1V; A0A0H2UKZ7), *Entamoeba histolytica* (PDB: 4NJO; Q76KF5), *Sulfolobus tokodaii* (PDB: 2EKL; Q972A9), *Pyrococcus horikoshii* (PDB: 1WWK; O50095) and *Lactobacillus plantarum* (PDB: 3EVT; F9URQ7). Type I PHGDHs from *M. tuberculosis* (PDB: 3DDN; P9WNX3), *Homo sapiens* (PDB: 2G76; O43175), *Pinus pinaster* (G3C901), *Zea mays* (B6SKK1), *Beta vulgaris* (A0A1Z4EAX4), *Oryza sativa* (Q6ZAA5), *Hordeum vulgare* (F2D807), *Macaca mulatta* (H9FST6), *Mustela putorius* (G9KGC8), *Rattus norvegicus* (O08651), *Bos taurus* (Q5EAD2), *Crotalus horridus* (T1DNB1), *Danio rerio* (Q6PHU9), *Callorhinchus milii* (V9KMU6), *Xenopus laevis* (A1L3N1), *Amblyomma aureolatum* (A0A1E1XAE4), *Hyalomma excavatum* (A0A131XJZ9), *Triatoma infestans* (A0A023F8D6), *Parasteatoda tepidariorum* (A0A2L2Y071), *Riptortus pedestris* (R4WJT3), *Chironex fleckeri* (D1FX73), *Agrobacterium radiobacter* (B9J9G2) and *Anabaena variabilis* (Q3M6M2).

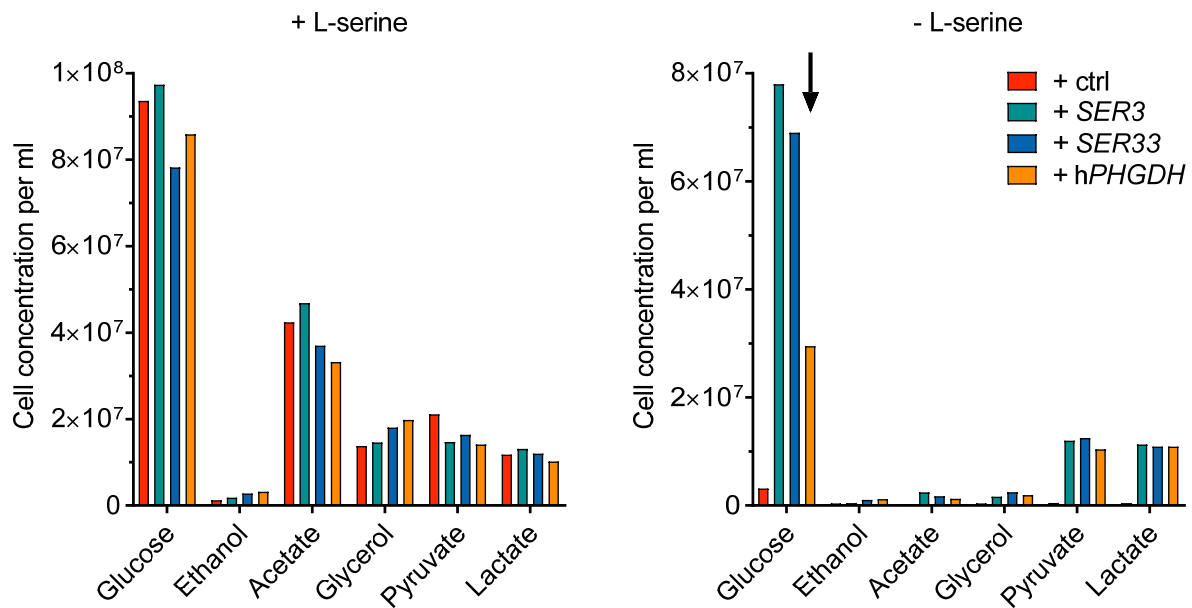


Supplemental Figure S4: **Validation of Ser3, Ser33, and human PHGDH expression in yeast rescue strains used in this study.** (A) SDS-PAGE and Western blot analysis of crude protein extracts of wild-type (BY4741 and FY4) strains, a *ser3* Δ *ser33* Δ strain transformed with an empty control plasmid (control), and the *SER3*, *SER33*, or *hPHGDH* rescue strains. For each protein extract, 5 μ l sample was loaded per well (except for the *hPHGDH* rescue strain, for which only 1 μ l extract was loaded). Recombinant purified his6-tagged Ser3 (1.09 μ g), Ser33 (2.46 μ g), and *hPHGDH* (0.04 μ g) were also analyzed for comparison (rSer3, rSer33, *rhPHGDH*). Expected

molecular weights (MW) of Ser3, Ser33, and *hPHGDH* were 51.1 kDa, 51.1 kDa, and 56.6 kDa for the native proteins (and 54.8 kDa, 54.8 kDa, and 58.7 kDa, respectively, for the corresponding His-tag fusion proteins). A primary antibody targeted against *hPHGDH* (#HTA994979, Sigma) was used for detection and exhibited cross reactivity with the yeast homologs Ser3 and Ser33, albeit with lower affinity. As observed previously [2], recombinant his6-Ser3 and his6-Ser33 migrated as multiple bands on an SDS-PAGE gel. *hPHGDH* protein expression was clearly detected in the *hPHGDH* rescue strain. Unspecific protein detection also occurred in the crude protein extracts, as indicated by the band detected in the *ser3Δser33Δ* control strain. A Blast search with the epitope sequence recognized by the PHGDH antibody against the *S. cerevisiae* genome suggests that in addition to Ser3 and Ser33, yeast glyoxylate reductases (Q02961, 45 kDa; P53839, 39 kDa) could react with this antibody. (B) qRT-PCR analysis revealed an at least 25- and 144-fold increased expression for *SER3* and *SER33*, respectively, in the corresponding rescue strains compared to a BY4741 wild-type control strain, while no expression of these genes could be detected in the empty vector *ser3Δser33Δ* control strain. –Fold changes were determined using the $2^{-\Delta\Delta C_t}$ method with either *ALG9* or *TFC1* as the reference genes. Values shown are means \pm SD of 2 biological and 2 technical replicates. Human *PHGDH* expression was also detected by qRT-PCR in the corresponding rescue strain (but not in the other strains), at considerably higher levels than the two reference genes used, based on C_t value comparison (not shown).



Supplemental Figure S5: **Expression of *SER3*, *SER33*, and human *PHGDH* enables colony formation of a *ser3Δser33Δ* double mutant strain in the absence of serine on respiratory carbon sources.** A *ser3Δser33Δ* double mutant strain (isogenic to BY4741) was transformed with the pAG416-GPD plasmid containing the bacterial *ccdB* gene (control strain) or the coding sequence of either *SER3*, *SER33*, or human *PHGDH* and spotted on agar plates prepared from a complex medium (A) or a chemically defined minimal medium containing the indicated carbon source and ammonium sulfate as a nitrogen source, but no uracil (for plasmid maintenance) (B—E). L-serine was added or not at a final concentration of 400 mg/l. Pre-cultures of all strains were diluted to an optical density of 3, 2, or 1 in sterile water and further diluted logarithmically before spotting (see panel A, right).



Supplemental Figure S6: **Human *PHGDH* expression cannot fully rescue growth of a *ser3Δser33Δ* double mutant strain on a fermentative carbon source.** A *ser3Δser33Δ* double mutant strain (isogenic to BY4741) was transformed with the pAG416-GPD plasmid containing

the bacterial *ccdB* gene (control strain) or the coding sequence of either *SER3*, *SER33*, or human *PHGDH* and cultivated in shaking flasks. Cultivations were performed in chemically defined minimal medium containing the indicated carbon source and ammonium sulfate as the nitrogen source, but no uracil (for plasmid maintenance). L-serine was added or not at a final concentration of 400 mg/l. Biomass was determined after 96 hours using a Beckman Coulter Multisizer Z3 as described in the Supplemental Methods section.