

**Applied Microbiology and Biotechnology**

**Supplementary Material**

**Increased biosynthesis of acetyl-CoA  
in the yeast *Saccharomyces cerevisiae*  
by overexpression of a deregulated pantothenate kinase gene**

Judith Olzhausen<sup>1</sup>, Mathias Grigat, Larissa Seifert<sup>2</sup>,  
Tom Ulbricht and Hans-Joachim Schüller

Center for Functional Genomics of Microbes,  
Abteilung Molekulare Genetik und Infektionsbiologie,  
Universität Greifswald,  
Felix-Hausdorff-Strasse 8, D-17487 Greifswald, Germany

**For correspondence:**

Hans-Joachim Schüller, E-mail: [schuell@uni-greifswald.de](mailto:schuell@uni-greifswald.de); Tel. +49 3834 4205703;  
Fax +49 3834 4205709.

Present address:

<sup>1</sup> Cendres+Métaux SA, CH-2501 Biel/Bienne, Switzerland

<sup>2</sup> Universitätsklinikum Hamburg-Eppendorf, Medizinische Klinik, Nephrologie

**Supplementary Table S1: Complete list of *S. cerevisiae* strains.**

Strain	Genotype
JS91.15-23	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3</i>
JS91.14-24	<i>MAT<math>\alpha</math> ura3 his3 cab1<sup>ts, G351S</sup></i>
JS19.1	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 cab1<math>\Delta</math>::HIS3 [pJO57: ARSH4 CEN6 URA3 CAB1]</i>
LSY20	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 cab4<math>\Delta</math>::HIS3 [pGE7: ARSH4 CEN6 URA3 CAB4]</i>
LSY21	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 cab5<math>\Delta</math>::HIS3 [pGE9: ARSH4 CEN6 URA3 CAB5]</i>
BY4741	<i>MAT<math>\alpha</math> ura3 leu2 his3 met15</i>
MGY5 (derived from JS91.15-23)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i>
MGY12 (derived from MGY5)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i>
MGY14 (derived from MGY12)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i>
MGY16 (derived from MGY14)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub> ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i>
MGY18 (derived from MGY16)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub> ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub> ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i>
MGY20 (derived from MGY16)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub> ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub> ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1-CYC1<sub>TER</sub></i>
MGY22 (derived from MGY18)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub> ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub> ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub> CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i>
MGY24 (derived from MGY20)	<i>MAT<math>\alpha</math> ura3 leu2 trp1 his3 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub> ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub> MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub> ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub> ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1-CYC1<sub>TER</sub> CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i>
MGY6 (derived from BY4741)	<i>MAT<math>\alpha</math> ura3 leu2 his3 met15 + pSH62 (2<math>\mu</math>m HIS3 GAL1-cre) HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i>

MGY13 (derived from MGY6)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i>
MGY15 (derived from MGY13)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i>
MGY17 (derived from MGY15)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i>
MGY19 (derived from MGY17)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i>
MGY21 (derived from MGY17)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1-CYC1<sub>TER</sub></i>
MGY23 (derived from MGY19)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i> <i>CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i>
MGY25 (derived from MGY21)	<i>MATa ura3 leu2 his3 met15 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1-CYC1<sub>TER</sub></i> <i>CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i>
TUY1 (derived from JS91.15-23)	<i>MATα ura3 leu2 trp1 his3 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i>
TUY4 (derived from MGY22)	<i>MATα ura3 leu2 trp1 his3 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i> <i>CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i> <i>GAT1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-FEN2-CYC1<sub>TER</sub></i>
LRY1 (derived from MGY22)	<i>MATα ura3 leu2 trp1 his3 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i> <i>CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i>

	<i>pcd1Δ::LEU2</i>
LRY2 (derived from TUY4)	<i>MATα ura3 leu2 trp1 his3 + pSH62 (2μm HIS3 GAL1-cre)</i> <i>HIP1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub></i> <i>ENO1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub></i> <i>MRH1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub></i> <i>ADH3::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub></i> <i>ZPS1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub></i> <i>CUP9::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa 260-495</sub>-CYC1<sub>TER</sub></i> <i>GAT1::TPI1<sub>PRO</sub>-HA<sub>3</sub>-FEN2-CYC1<sub>TER</sub></i> <i>pcd1Δ::LEU2</i>

**Supplementary Table S2: Plasmids used and constructed in this work.**

Plasmid	Genetic markers
pRS415	<i>ARSH4 CEN6 LEU2</i> (Sikorski and Hieter, 1989)
pRS416	<i>ARSH4 CEN6 URA3</i> (Sikorski and Hieter, 1989)
p415-MET25	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub></i> (Mumberg <i>et al.</i> , 1994)
p416-MET25	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub></i> (Mumberg <i>et al.</i> , 1994)
p426-MET25HA	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub></i> (Mumberg <i>et al.</i> , 1994)
pSH62	2μm <i>HIS3 GAL1-cre</i> (Güldener <i>et al.</i> , 1996)
pTM8	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub>-CAB1</i> (wild-type)
pLS12-S8	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub>-CAB1<sup>G351S W331L</sup></i>
pLS12-S9	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub>-CAB1<sup>G351S D114E</sup></i>
pLS12-S10	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub>-CAB1<sup>G351S F103V</sup></i>
pLS12-Y6	<i>ARSH4 CEN6 URA3 MET25<sub>PRO</sub>-CAB1<sup>G351S A22G</sup></i>
pJO20	<i>CAB1</i>
pJO57	<i>ARSH4 CEN6 URA3 CAB1</i>
pKH45	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1</i>
pKH46	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>W331L</sup></i>
pKH48	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>Y326A F330A</sup></i>
pKH49	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>I234E</sup></i>
pKH50	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>N155V</sup></i>
pKH51	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>S158V</sup></i>
pKH52	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>R173A</sup></i>
pKH53	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>A233E</sup></i>
pKH54	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-CAB1<sup>W331R</sup></i>
pSBS5	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1</i> (Olzhausen <i>et al.</i> , 2009)
pJO3	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB3</i>
pJO73	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-PANK3</i> (Olzhausen <i>et al.</i> , 2009)
pEB5	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sup>Y326A F330A</sup></i>
pEB6	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-cab1<sup>I234E</sup></i>
pEB8	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sup>W331L</sup></i>
pEB22	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sup>N155V</sup></i>
pEB23	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-cab1<sup>S158V</sup></i>
pEB25	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-cab1<sup>R173A</sup></i>
pEB26	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sup>A233E</sup></i>
pEB27	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sup>W331R</sup></i>
pGE7	<i>ARSH4 CEN6 URA3 CAB4</i>
pGE8	<i>ARSH4 CEN6 LEU2 CAB4</i>
pGE9	<i>ARSH4 CEN6 URA3 CAB5</i>
pGE10	<i>ARSH4 CEN6 LEU2 CAB5</i>
pLS14	2μm <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-hCOASY</i> (full-length)

pLS15	2 $\mu$ m <i>URA3 MET25<sub>PRO</sub>-HA<sub>3</sub>-hCOASY</i> (aa 30-564)
pLS20	<i>ARSH4 CEN6 LEU2 MET25<sub>PRO</sub>-hCOASY</i> (full-length)
pSBS7	<i>cab1<math>\Delta</math>::HIS3</i> (Olzhausen <i>et al.</i> , 2009)
pSB2	<i>cab4<math>\Delta</math>::HIS3</i> (Olzhausen <i>et al.</i> , 2009)
pSB5	<i>cab5<math>\Delta</math>::HIS3</i> (Olzhausen <i>et al.</i> , 2009)
pLR2	<i>pcd1<math>\Delta</math>::LEU2</i>
pLEUTEX3	<i>TPI1<sub>PRO</sub>-HA<sub>3</sub>-CYC1<sub>TER</sub>::loxP::LEU2::loxP</i>
pLEUTEX-CAB1	<i>ZPS1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1-CYC1<sub>TER</sub>::ZPS1-3'-flank</i>
pLEUTEX-CAB1 W331R	<i>ZPS1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB1<sub>W331R</sub>-CYC1<sub>TER</sub>::ZPS1-3'-flank</i>
pLEUTEX-CAB2	<i>HIP1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB2-CYC1<sub>TER</sub>::HIP1-3'-flank</i>
pLEUTEX-CAB3	<i>ENO1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB3-CYC1<sub>TER</sub>::ENO1-3'-flank</i>
pLEUTEX-CAB4	<i>MRH1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB4-CYC1<sub>TER</sub>::MRH1-3'-flank</i>
pLEUTEX-CAB5	<i>ADH3-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-CAB5-CYC1<sub>TER</sub>::ADH3-3'-flank</i>
pLEUTEX-HAL3	<i>CUP9-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-HAL3<sub>aa260-495</sub>-CYC1<sub>TER</sub>::CUP9-3'-flank</i>
pLEUTEX-FEN2	<i>GAT1-5'-flank::TPI1<sub>PRO</sub>-HA<sub>3</sub>-FEN2-CYC1<sub>TER</sub>::GAT1-3'-flank</i>

aa, amino acids; PRO, promoter; TER, terminator.

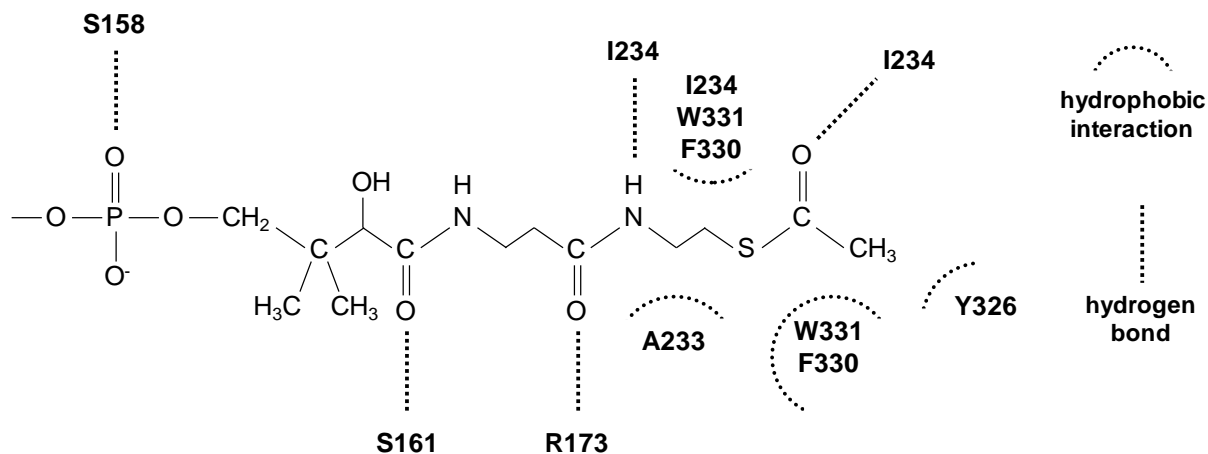
### Supplementary Table S3: Oligonucleotides used in this work.

Name	Gene	Position	Sequence (5'-3')
<b>ORF-Primer</b>			
CAB1-Bam-Start	<i>CAB1</i>	+1/+19	gactggatccATGCCGCGAATTACTCAAG
CAB1-Hind-Stop	<i>CAB1</i>	+1104/+1084	gactaagcttCTACGTA CTTGTTTTCTTAGT
YIL083C-BamHI	<i>CAB2</i>	+1/+20	gactggatccATGCCACCTCTACCCGTGCT
YIL083C-XhoI	<i>CAB2</i>	+1098/+1075	gactctcgagTTATTTTCGTAGCTAGCTTAGTTTT
YKL088W-Start	<i>CAB3</i>	+1/+20	gactggatccATGACGGATGAAAAAGTGAA
YKL088W-Stop	<i>CAB3</i>	+1716/+1693	gactaagcTTAAACTTGCGTTTTTACGTCCTC
CAB4-Bam-Start	<i>CAB4</i>	+1/+20	gactggatccATGGTTGAGGAAAATTCCAG
CAB4-Hind-Stop	<i>CAB4</i>	+918/+897	gactaagcTTATACGCAAGGGTTTTGTGGA
CAB5-Bam-Start	<i>CAB5</i>	+1/+20	gactggatccATGCTGGTAGTGGGATTGAC
CAB5-Cla-Stop	<i>CAB5</i>	+726/+705	gactatcgatTTATACCGCTGAAGACTTTTTTA
Hal3-BgIII Start260	<i>HAL3</i>	+778/+798	gatcagatctAAAATGGATGATGGAAAATTGCACGTG
Hal3-HindIII Stop495	<i>HAL3</i>	+1485/+1464	gatcaagcttTAGTTATTTTTTTGGGTACCCACCC
FEN2-BamHI Start	<i>FEN2</i>	+1/+22	gatcggatccATGATGAAGGAATCGAAATCTA
FEN2-HindIII Stop	<i>FEN2</i>	+1539/+1517	gatcaagcttCTATCGGAAAGGATTTGAAATTT
<b>Mutagenic primer</b>			
CAB1-	<i>CAB1</i>	+445/+483	TACCCATACCTTCTAGTC <b>GT</b> GATAGGGT-

N155V-F			CGGGTGTCTCA
CAB1-N155V-R	CAB1	+483/+445	TGAGACACCCGACCCTAT <b>CAC</b> ACTAGAGAGGTATGGGTA
CAB1-S158V-F	CAB1	+453/+489	CCTTCTAGTCAATATAGGG <b>GTGG</b> GTGTC-TCAATATTA
CAB1-S158V-R	CAB1	+489/+453	TAATATTGAGACACCC <b>CACC</b> CCTATATTGACTAGAAGG
CAB1-R173A-F	CAB1	+499/+537	GAACCAAACAATTTTAGT <b>GCCG</b> TAGGCG-GTTCTTCACTG
CAB1-R173A-R	CAB1	+537/+499	CAGTGAAGAACC GCCTAC <b>GGC</b> ACTAAAA-TTGTGGTTTC
CAB1-I234E-F	CAB1	+682/+717	GGTCTAAAGTCGTCAGCT <b>GAGG</b> CAAGTT-CATTTGGT
CAB1-I234E-R	CAB1	+717/+682	ACCAAATGAACTTGC <b>CTC</b> AGCTGACGAC-TTTAGACC
CAB1-A233E-F	CAB1	+682/+716	GGTCTAAAGTCGTCAG <b>GAG</b> ATTGCAAGTT-CATTTGG
CAB1-A233E-R	CAB1	+716/682	CCAAATGAACTTGAAT <b>CTCT</b> GACGAC-TTTAGACC
CAB1-W331R-F	CAB1	+972/1013	GAGCTACGCTATTAATTTTT <b>CGG</b> TCACAA-GGATCAAAGCAAGC
CAB1-W331R-R	CAB1	+1013/972	CGTTGCTTTGATCCTTGTGAC <b>CCG</b> AAAATT-AATAGCGTAGCTC
CAB1-W331L-F	CAB1	+972/+1013	GAGCTACGCTATTAATTTTT <b>TG</b> TCACAA-GGATCAAAGCAAGC
CAB1-W331L-R	CAB1	+1013/+972	CGTTGCTTTGATCCTTGTGAC <b>CA</b> AAAAATT-AATAGCGTAGCTC
CAB1-Y326A F330A-F	CAB1	+958/+1008	ACCATGAACACTTTGAGC <b>GCGG</b> CTATTAAT- <b>GCG</b> TGGTCACAAGGATCAAAG
CAB1-Y326A F330A-R	CAB1	+1008/+958	CTTTGATCCTTGTGACC <b>CGC</b> AATAATAGC- <b>CGC</b> GCTCAAAGTGTTTCATGGT
<b>Primers for amplification of flanking regions to be inserted into pLEUTEX cassettes</b>			
ADH3 5F Sacl	ADH3	-2100/-2081	gatcgagctcCTGCATGTTTACAAGGCCAAA
ADH3 5F BglII	ADH3	-1631/-1650	gatcagatctTGGTCAAGACCAGAATTACT
ADH3 3F NheI	ADH3	-1550/-1531	gatcgctagcCAAGGAGTAGCTTCCTTCAA
ADH3 3F KpnI	ADH3	-1131/-1150	gatcggtagcCATTGTGCAAAGACCCATGC
ADH3 Veri	ADH3	-2140/-2121	CAGAAGCTTTCGTGAGATAC
CUP9 5F Sacl	CUP9	-1570/-1551	gatcgagctcGACGGCTTGTAAACGGGTGCC
CUP9 5F BglII	CUP9	-1141/-1160	gatcagatctAGGGGCCCCGACAATATTGCT
CUP9 3F NheI	CUP9	-1030/-1011	gatcgctagcGTAGTATGCCTCCGAAGCTC
CUP9 3F KpnI	CUP9	-601/-620	atcggtagcGTGCTCAGAAAACCCCGTGG
CUP9 Veri	CUP9	-1620/-1601	GATATTTGAATCAGCTGGAG
ENO1 5F Sacl	ENO1	-1580/-1561	gatcgagctcTACATTCATGTTTGCACGCT
ENO1 5F BglII	ENO1	-1231/-1250	gatcagatctTCTGGATAAGTGCTGTGCAT
ENO1 3F	ENO1	-1106/-1086	gatcgctagcAATGGCCCTATATTGAAAGC

NheI			
ENO1 3F KpnI	<i>ENO1</i>	-771/-790	gatcgggtaccGCTCAAGTGCCCGCGGCATC
ENO1 Veri	<i>ENO1</i>	-1210/-1191	TGACTCAAATTCTTCTCGAC
GAT1 5F SacI	<i>GAT1</i>	+1881/+1900	gatcgagctcAACCGAGACCTGAACATCCT
GAT1 5F BamHI	<i>GAT1</i>	+2320/+2301	gatcggatccAGCGGGAAACTCCTTAGTGT
GAT1 3F NheI	<i>GAT1</i>	+2451/+2460	gatcgctagcGCCCCAAATGTCAGTGATGG
GAT 3F KpnI	<i>GAT1</i>	+2870/+2851	gatcgggtaccAGAAACGATATTAAGCCTGG
GAT1 Veri	<i>GAT1</i>	+1811/+1830	CTAAACCAGTTATCCTAGGC
HIP1 5F SacI	<i>HIP1</i>	-1573/-1554	gactgagctcTTGTCTGCCGAAATTCTGTG
HIP1 5F BamHI	<i>HIP1</i>	-1162/-1171	gactggatccAGTAGCTTGTGAACGATAGG
HIP1 3F NheI	<i>HIP1</i>	-960/-941	gactgctagcTTTACCCCTCTCCACAGATC
HIP1 3F KpnI	<i>HIP1</i>	-636/-655	gactgggtaccCCAGGTTGAATACAAAACGG
HIP1 5-Veri	<i>HIP1</i>	-1620/-1601	ATTGAGCCACCGCTTCATCT
MRH1 5F SacI	<i>MRH1</i>	-1860/-1841	gatcgagctcTCTTGTTCCCATCTACGGGT
MRH1 5F BglII	<i>MRH1</i>	-1471/-1490	gatcagatctGGAAGCGTTGGAAAAGAAGG
MRH1 3F NheI	<i>MRH1</i>	-1230/-1211	gatcgctagcCTCCTCGAGACAAGGTCGCT
MRH1 3F KpnI	<i>MRH1</i>	-851/-870	gatcgggtaccGTTTGGGTCTCTTGGTAACC
MRH1 Veri	<i>MRH1</i>	-1940/-1921	ACTTGCGCGCCGACATTTG
ZPS1 5F SacI	<i>ZPS1</i>	-2380/-2361	atcgagctcAGGCGCGTGTCTACCTCGTA
ZPS1 5F BglII	<i>ZPS1</i>	-1931/-1150	gatcagatctGGCCTCGGCAACTTTCAGGG
ZPS1 3F NheI	<i>ZPS1</i>	-1560/-1541	gatcgctagcGGTATGATCTACCCCATTCG
ZPS1 3F KpnI	<i>ZPS1</i>	-1241/-1250	gatcgggtaccCCGCGAAGACTACGCAAATA
ZPS1 Veri	<i>ZPS1</i>	-2430/-2411	AGTTAGTGGTTGCCTTCCGT
<b>Construction of pLEUTEX3</b>			
LEU2-LBN			gactggatccatgcatCAACCCTTAATATAACTTCGTAT A
LEU2-RXN			gactctcgaggctagcACCTAATAACTTCGTATAGCAT
MET25HAX			gacttctagaAAAATGTATCCTTATGATGTTCC
CYC1TER NSI			gactatgcatCGGCCGCAAATTAAGCCTT
TPI1-Prom	<i>TPI1</i>	-600/-580	gactgagctcagatctGAGACCTAACTACATAGTGT
TPI1PR-XB	<i>TPI1</i>	-26/-5	gacttctagaGTTTATGTATGTGTTTTTTGT

Authentic gene sequences are shown in capital letters; auxiliary sequences are depicted in lower case letters. Bold letters indicate the sequence of mutations introduced.

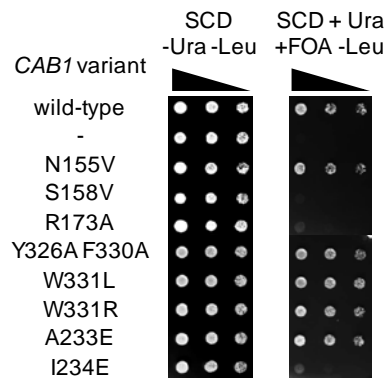


**Supplementary Figure S1:** Presumed binding of acetyl-CoA to the substrate binding site of yeast PanK (derived from the crystal structure of human PanK3 isoenzyme in the presence of acetyl-CoA; taken from Hong *et al.* [2007] and assigned to the yeast enzyme)



Cab1 (Sc) MPRITQEISYNCDYGDNTFNLAIDIGGTLAKVVFSPiHSNRLMFYTIETEKIDKFMELLH 60  
 CoaA (Sa) -----MKVGI**DAGGTLIKIVQE**--QDNQRTFKTELTKNIDQVVEWLN 40  
  
 Cab1 (Sc) SIIKEHNNGCYRMTHIIATGGGAFKFYDLLYENFPQIKGISRFEE**MEGLIHGLDFFIHEI** 120  
 CoaA (Sa) QQQ-----IEKLCLTGGNAGVIAENINIP-----AQIFVEFDAASQGLGILLKEQ 85  
  
 Cab1 (Sc) PDEVFTYNDQDGERIIP**TSSGT**MDSKAIYPYLLV**NI**SGV**SILK**VTEPN**NFSRV**GGSSLG 180  
 CoaA (Sa) G-----HDLADYIFANVGTG**TSLHY**FDGQ-SQRRVGGIGTG 120  
  
 Cab1 (Sc) GGT**LWGLLSLITGAQTYDQMLDWAQEGDNSSVDMLVGD**IYGT**DYNKIGLKSSA**TIASSFGK 240  
 CoaA (Sa) GGM**IQGLGYLLSQITDYKQLTDM**AQHGRNTIDLKVRHIY-KDTE-PP**IPGDLTAA**NFGH 178  
  
 Cab1 (Sc) VFQNRMTSNKSLENNENKLYSSHESIEK**NNGQMFKNPDICKSLLFAIS**NNIGQIAYLQAK 300  
 CoaA (Sa) VLHHL**DADFTPSN**-----KLA**AVIGV**VEVVT**TMAITVAR** 213  
  
 Cab1 (Sc) INNIQNIYFGGSYTRGHLTTMNTLS**YAIN**EW**SQ**SKQAFFLKHEGYLGAMGAFLSASRHS 360  
 CoaA (Sa) EFKTENIVYIGSS**FHNNALLR**KVVEDYTVLRG---CKPYYVENGAFSGAIGALYLEK\* 267  
  
 Cab1 (Sc) STKKTST\* 367

**Supplementary Figure S2:** Similarity of pantothenate kinases from *Saccharomyces cerevisiae* (Sc; *CAB1* gene product) and *Staphylococcus aureus* (Sa; *coaA* gene product). Conserved amino acids are shaded in grey. Amino acids used for mutational analysis of Cab1 are indicated in bold and by inverse shading.



**Supplementary Figure S3:** Functional analysis of *CAB1* variants by plasmid shuffling. Strain JS19.1 with a chromosomal *cab1* $\Delta$  null allele and *ARS CEN URA3 CAB1* rescue plasmid (pJO57) was transformed with *ARS CEN LEU2* plasmids containing the *CAB1* variants indicated. All transformants were able to grow on double-selective synthetic medium (SCD-Ura-Leu) while only functional variants of *CAB1* allowed growth on FOA-containing medium after loss of the rescue plasmid. FOA: 5-Fluoroorotic acid.

## Supplementary description: Construction of pLEUTEX3

Plasmid pLEUTEX3 was constructed in a three-step-procedure:

### 1. Insertion of selection marker *LEU2* into pBluescript

Plasmid pJS491 (unpublished) had been constructed by inserting the selection marker *LEU2* flanked by loxP sites into the backbone of pUC19. Using pJS491 as a template together with PCR primers LEU2-LBN (introduces restriction sites for *Bam*HI and *Nsi*I) and LEU2-RXN (introduces restriction sites for *Xho*I and *Nhe*I), a 1.73 kb loxP::*LEU2*::loxP fragment was amplified, digested with *Bam*HI + *Xho*I and inserted into *Bam*HI + *Xho*I cleaved pBluescript II KS(+) to give pLEUTEX1.

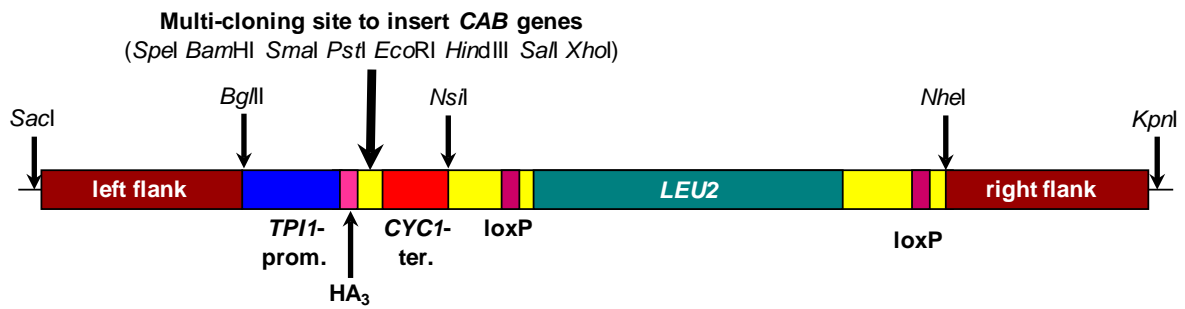
### 2. Insertion of the HA<sub>3</sub> epitope and *CYC1* terminator

Using p425-MET25HA as a template together with PCR primers MET25HAX (introduces restriction site for *Xba*I) and *CYC1*TERNSI (introduces restriction site for *Nsi*I), a 0.4 kb fragment was amplified. This PCR fragment was digested with *Xba*I + *Nsi*I and inserted into pLEUTEX1 which had been also digested with these restriction enzymes (giving pLEUTEX2).

### 3. Insertion of *TPI1* promoter

The *TPI1* promoter was amplified using chromosomal DNA as a template together with PCR primers TPI1-Prom (introduces restriction sites for *Sac*I + *Bgl*II) and TPI1PR-XB (introduces restriction site for *Xba*I). The resulting 0.6 kb fragment was digested with *Sac*I + *Nsi*I and inserted into pLEUTEX2 which had been digested with the same restriction enzymes (to give pLEUTEX3).

Using pLEUTEX3, gene-specific expression cassettes can be constructed by inserting the coding region of a CoA biosynthetic gene (*TPI1-CAB* fusion) and ligating flanking sequences (about 400 bp; left flank: *Sac*I + *Bgl*II fragment, right flank: *Nhe*I + *Kpn*I fragment) on both sides. Following release of the expression cassette by cleavage with *Sac*I + *Kpn*I, recombination can occur at the chromosomal site homologous to the flanking region.



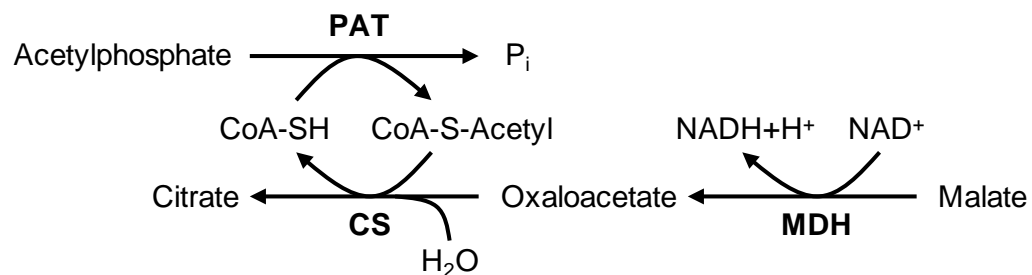
**Supplementary Figure S4:** Functional elements of pLEUTEX expression cassettes suitable for repeated integration of CoA biosynthetic genes. Selection marker *LEU2* flanked by loxP sites can be removed after a successful transformation by induction of cre recombinase which targets loxP sites. Several unique restriction sites following the *TPI1*-HA promoter/epitope segment allow insertion of *CAB* reading frame cassettes. For integration of the expression/selection cassette by homologous recombination, flanking sequences from a genomic site of choice must be inserted by using restriction sites *SacI* + *BglII* (left flank) and *NheI* + *KpnI* (right flank), respectively. Prior to yeast transformation, expression cassettes together with flanking regions can be released by cleavage with *SacI* + *KpnI*.

### Supplementary Method: Enzymatic assay of CoA + acetyl-CoA concentrations

The cycling assay described by Bergmeyer (1974) allows repeated use of CoA and acetyl-CoA by regeneration of CoA to acetyl-CoA catalyzed by phosphotransacetylase (PAT), leading to signal amplification. Using the citrate synthase (CS) reaction, acetyl-CoA and oxaloacetate react to give citrate. Oxaloacetate is synthesized from malate by NAD-dependent malate dehydrogenase which serves as a preceding indicator reaction, leading to an increase of absorbance by NADH at 340 nm.

NEM (N-ethylmaleimide) can react with free SH group of CoA so that only acetyl-CoA is detected.

Various samples containing CoA + acetyl-CoA with known concentrations (ratio 1:1) were used for calibration and validation of CoA assays in protein-free metabolite extracts. Dilution of these extracts as a result of protein removal was considered by calculation of the dilution factor.



#### Reaction mixture (final concentrations):

- 140 mM Triethanolamine/HCl
- 10.5 mM Malate (potassium salt)
- 0.46 mM DTT or 0.46 mM NEM
- 1.4 mM NAD
- 4.6 mM Acetylphosphate (lithium salt)
- 7000 U/l PAT
- 1300 U/l CS
- 9800 U/l MDH