

FIG S1 Impact of *tpo1Δ* and *pdr12Δ* mutations on tolerance of *S. cerevisiae* to pimelic acid, sorbate, and spermidine | *S. cerevisiae* strains were pregrown on liquid SMD and spotted on plates containing SMD (pH 4.5) **(A)** or in modified SMD (pH 4.5) in which the Mg²⁺ concentration was reduced to 50 μM to facilitate polyamine uptake **(B)**. Plates were supplemented with 50 μM, 150 μM, 1 mM, 1.5 mM, 2 mM or 3 mM pimelic acid, 0.5 mM potassium sorbate, 6 mM spermidine or water (control) as indicated. Strains and relevant genotypes are indicated on the left-hand side, estimated numbers of plated cells are indicated on top and incubation times at 30°C are indicated below the photographs.

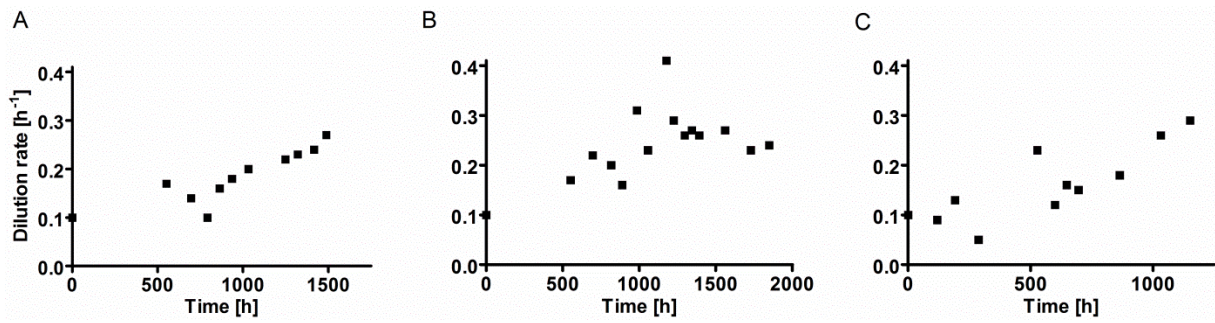


FIG S2 Laboratory evolution of *S. cerevisiae* CEN.PK113-7D cells in accelerostat cultures for improved growth in biotin-free synthetic media | (A) IMS0478 (B) IMS0480 (C) IMS0481.

Dilution rates were automatically feed-back controlled based on continuous analysis of the CO₂ output and the medium feed rate (see Methods). Dilution rates [h⁻¹] are shown for three independent experiments.

TABLE S1 Mutations in evolved biotin prototrophic *S. cerevisiae* strains | Single nucleotide variants were called by comparison of whole-genome sequence data from the four strains evolved for biotin prototrophy with their parent strain CEN.PK113-7D. Listed are all nonsynonymous and nonsense mutations (*). Descriptions of gene functions were obtained from the *Saccharomyces* Genome Database website (www.yeastgenome.org).

Gene	Amino acid change	Nucleotide change	Description
IMS0478			
<i>ULS1</i>	Asp-130-Val	A389T	Swi2/Snf2-related translocase required for maintenance of NHEJ inhibition at telomeres. Plays role in antagonizing silencing during mating-type switching.
<i>GPI11</i>	Val-11-Gly	T32G	ER membrane protein involved in a late step of GPI anchor assembly. Involved in the addition of phosphoethanolamine to the multiply mannosylated glycosylphosphatidy-linositol (GPI) intermediate.
<i>VIK1</i>	Leu-577-Ile	T1729A	Protein that forms a kinesin-14 heterodimeric motor with Kar3p. Required for sister chromatid cohesion.
<i>NMD2</i>	Ser-567-*	C1700A	Protein involved in the nonsense-mediated mRNA decay (NMD) pathway. Involved in telomere maintenance.
<i>THO2</i>	Ser-53-*	C158A	Subunit of the THO complex. THO is required for efficient transcription elongation and involved in transcriptional elongation-associated recombination.
<i>HSE1</i>	Val-222-Ile	G664A	Subunit of the endosomal Vps27p-Hse1p complex. Required for sorting of ubiquitinated membrane proteins, as well as for recycling of Golgi proteins and formation of luminal membranes.
IMS0480			
<i>MAK1</i> <i>0</i>	Pro-42-Leu	C125T	Non-catalytic subunit of N-terminal acetyltransferase of the NatC type. Required for replication of dsRNA virus; expression is glucose-repressible.
<i>TPO1</i>	Asp-262-Gly	A785G	Polyamine transporter of the major facilitator superfamily. Member of the 12-spanner drug-H(+) antiporter DHA1 family. Recognizes spermine, putrescine, and spermidine. Catalyzes uptake of polyamines at alkaline pH and excretion at acidic pH.
<i>ALD4</i>	Gly-400-Ser	G1198A	Mitochondrial aldehyde dehydrogenase. Required for growth on ethanol and conversion of acetaldehyde to acetate.

<i>BNI4</i>	Ser-835-Pro	T2503C	Targeting subunit for Glc7p protein phosphatase. Localized to the bud neck, required for localization of chitin synthase III to the bud neck via interaction with the chitin synthase III regulatory subunit Skt5p.
<i>WAR1</i>	Glu-456-*	G1366T	Homodimeric Zn ₂ Cys ₆ zinc finger transcription factor. Binds to a weak acid response element to induce transcription of <i>PDR12</i> and <i>FUN34</i> , encoding an acid transporter and a putative ammonia transporter, respectively.
<i>CYR1</i>	Glu-1879-Asp	A5673C	Adenylate cyclase. Required for cAMP production and cAMP-dependent protein kinase signalling.
<i>BIO5</i>	Ile-145-Thr	T434C	Putative transmembrane protein involved in the biotin biosynthesis. Responsible for uptake of 7-keto 8-aminopelargonic acid.
IMS0481			
<i>ASA1</i>	Ile-436-Val	A1306G	Subunit of the ASTRA complex, involved in chromatin remodelling. Telomere length regulator involved in the stability or biogenesis of PIKKs such as TORC1.
<i>TPO1</i>	Asp-133-Asn	G397A	Polyamine transporter of the major facilitator superfamily. Member of the 12-spanner drug-H(+) antiporter DHA1 family. Recognizes spermine, putrescine, and spermidine. Catalyses uptake of polyamines at alkaline pH and excretion at acidic pH.
<i>VPS15</i>	Phe-452-Cys	T1355G	Serine/threonine protein kinase involved in vacuolar protein sorting. Functions as a membrane-associated complex with Vps34p.
<i>SPT21</i>	Glu-581-Lys	G1741A	Protein with a role in transcriptional silencing. Required for normal transcription at several loci including HTA2-HTB2 and HHF2-HHT2, but not required at the other histone loci.
<i>VHT1</i>	Gly-288-Ala	G863C	High-affinity plasma membrane H ⁺ -biotin (vitamin H) symporter. Mutation results in fatty acid auxotrophy.
	Asn-174-Lys	T522G	
<i>BCY1</i>	Val-262-Phe	G784T	Regulatory subunit of the cyclic AMP-dependent protein kinase (PKA). PKA is a component of a signalling pathway that controls a variety of cellular processes, including metabolism, cell cycle, stress response, stationary phase, and sporulation.
<i>ALP1</i>	Phe-422-Ser	T1265C	Arginine transporter. Expression is normally very low and it is unclear what conditions would induce significant expression.
<i>PDE1</i>	Gly-41-Cys	G121T	Low-affinity cyclic AMP phosphodiesterase. Controls glucose and intracellular acidification-induced cAMP signalling, target of the cAMP-protein kinase A (PKA) pathway.

<i>PDR12</i>	Trp-765-*	G2295A	Plasma membrane ATP-binding cassette (ABC) transporter. Weak-acid-inducible multidrug transporter required for weak organic acid resistance. Induced by sorbate and benzoate and regulated by War1p.
<i>CIT2</i>	Ala-289-Val	C866T	Citrate synthase, peroxisomal isozyme involved in glyoxylate cycle. Catalyzes condensation of acetyl coenzyme A and oxaloacetate to form citrate.
<i>REC10</i> 4	Ser-76-Tyr	C227A	Protein involved in early stages of meiotic recombination. Required for meiotic crossing over.
IMS0496			
<i>CSS3</i>	Asp-117-Tyr	G349T	Protein of unknown function, secreted when constitutively expressed. Deletion mutants are viable and have elevated levels of Ty1 retrotransposition and Ty1 cDNA.
<i>TPO1</i>	Trp-302-*	G905A	Polyamine transporter of the major facilitator superfamily. Member of the 12-spanner drug-H(+) antiporter DHA1 family. Recognizes spermine, putrescine, and spermidine. Catalyzes uptake of polyamines at alkaline pH and excretion at acidic pH.
<i>TDA6</i>	Pro-38-Arg	C113G	Putative protein of unknown function.
<i>NCA3</i>	Ala-289-Gly	C866G	Protein involved in mitochondrion organization.
<i>RPN1</i>	Lys-943-*	A2827T	Non-ATPase base subunit of the 19S RP of the 26S proteasome.
<i>FCJ1</i>	Glu-234-Asp	A702C	Component of the MICOS complex. MICOS is a mitochondrial inner membrane complex that extends into the intermembrane space and has a role in the maintenance of crista junctions, inner membrane architecture, and formation of contact sites to the outer membrane.

TABLE S2 mRNA levels of *BIO* genes in strains evolved for biotin prototrophy grown in the presence (+) or absence (-) of biotin prior to mRNA extraction | (+) Relative expression levels of *BIO1*, *BIO2*, *BIO3*, *BIO4*, and *BIO6* measured in the parent strain CEN.PK113-7D and the evolved strains IMS0478, IMS0480, IMS0481, and IMS0496 upon growth in the presence of biotin supplementation. (-) Relative expression levels of *BIO1*, *BIO2*, *BIO3*, *BIO4*, and *BIO6* measured in the evolved strains IMS0478, IMS0480, IMS0481, and IMS0496 upon growth in the absence of biotin supplementation. The parent strain was unable to grow in absence of biotin. All qPCR experiments were carried out on duplicate cultures, with analytical triplicates for each culture. Relative expression levels were determined according to the $\Delta\Delta CT$ method (1) and indicated with the standard error of the mean (SEM) of duplicate analyses. A graphical representation of the data can be found in Fig. 3.

	Biotin	IMS0478	IMS0480	IMS0481	IMS0496	CEN.PK113-7D
<i>BIO1</i>	+	0.8 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.8 ± 0.1	0.1 ± 0.0
	-	16.2 ± 2.3	1.8 ± 0.7	2.7 ± 0.1	24.4 ± 0.3	-
<i>BIO2</i>	+	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
	-	2.2 ± 0.3	0.2 ± 0.1	0.6 ± 0.1	5.0 ± 0.5	-
<i>BIO3</i>	+	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	-	6.4 ± 0.7	0.0 ± 0.0	0.1 ± 0.0	1.1 ± 0.0	-
<i>BIO4</i>	+	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	-	4.8 ± 0.7	0.0 ± 0.0	0.6 ± 0.1	1.7 ± 0.1	-
<i>BIO6</i>	+	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
	-	5.2 ± 0.8	0.1 ± 0.0	0.6 ± 0.0	4.1 ± 0.0	-

1. **Schmittgen TD, Livak KJ.** 2008. Analyzing real-time PCR data by the comparative CT method. *Nat Protoc* **3**:1101-1108.