Supporting Information

Keller et al. 10.1073/pnas.1222607110

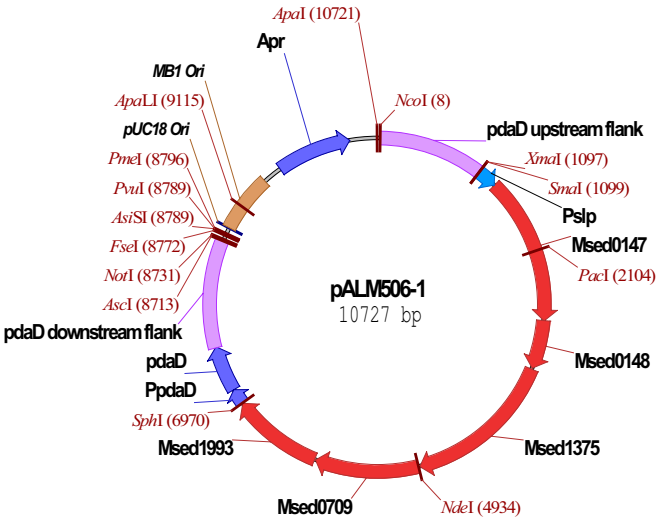

Fig. S1. Plasmid map of pALM506-1 used to transform the *Pyrococcus furiosus* arginine decarboxylase deletion strain ΔpdaD to generate strain PF506.

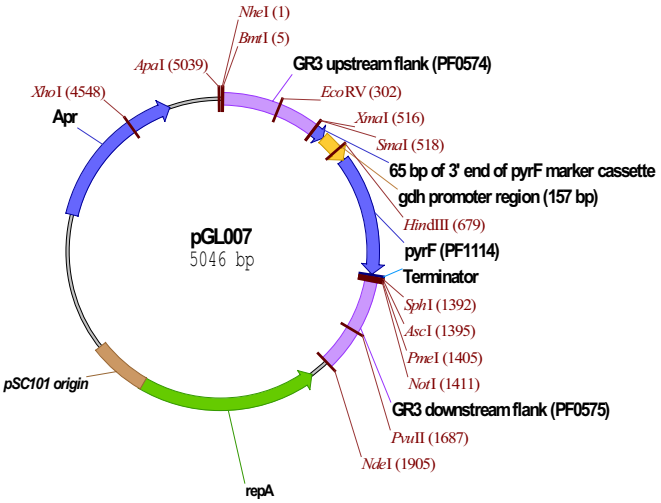

Fig. S2. Plasmid map of pGL007 vector targeting the region between the loci PF0574 and PF0575 in the *P. furiosus* genome.

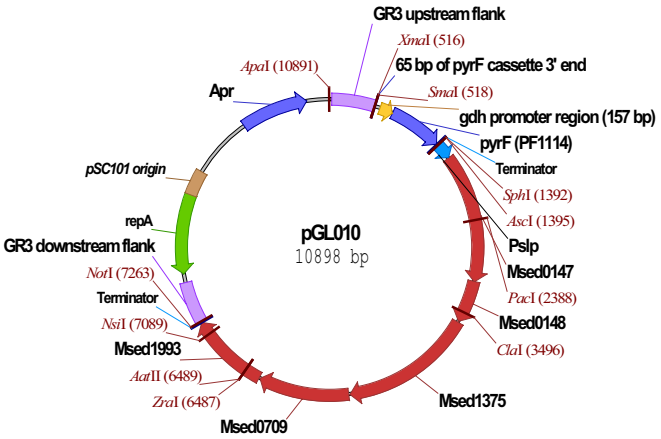

Fig. S3. Plasmid map of pGL010 used to transform *P. furiosus* strain COM1 to generate strain MW56.

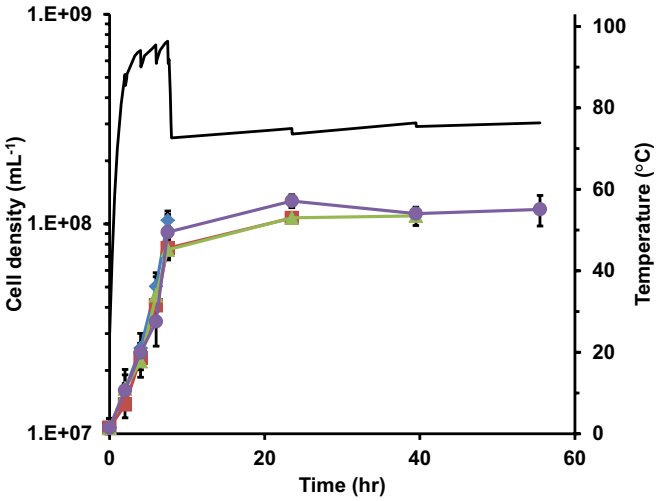

Fig. S4. Growth of *P. furiosus* strain PF506 at 98°C and subsequent temperature shift to 75°C. *P. furiosus* was grown in four 800-mL cultures at 98°C until the cell density reached 5×10^8 cells/mL The temperature (shown as black line) was then shifted to 75°C, and individual bottles were removed and harvested after 0 h (blue diamond), 16 h (red square), 32 h (green triangle), and 48 h (purple circle). The enzyme activities in each cell type are summarized in Fig. 2*B*.

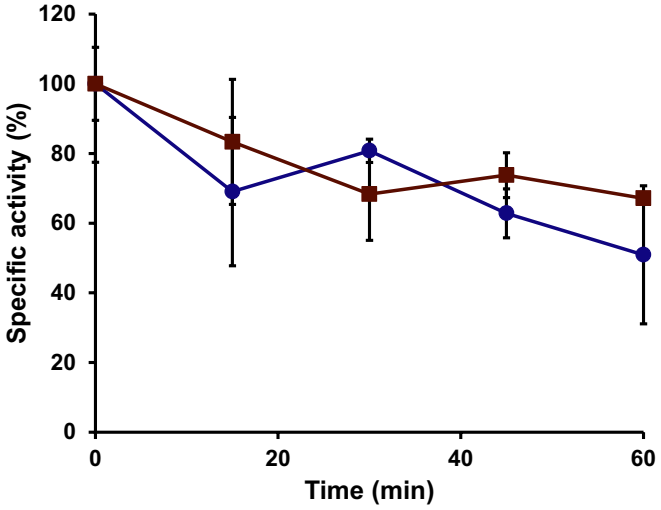

Fig. S5. Stability of E2 and E3 using an E2 + E3 coupled assay at 75°C after incubation at 90°C for the indicated amount of time in cell-free extracts of *P. furiosus* strain PF506 (blue circles) and of the endogenous *P. furiosus* glutamate dehydrogenase (red squares). The specific activity of E2+E3 in PF506 (grown at 72°C) is about twofold higher than that measured in *M. sedula*. Activity is expressed as percentage maximum activity.

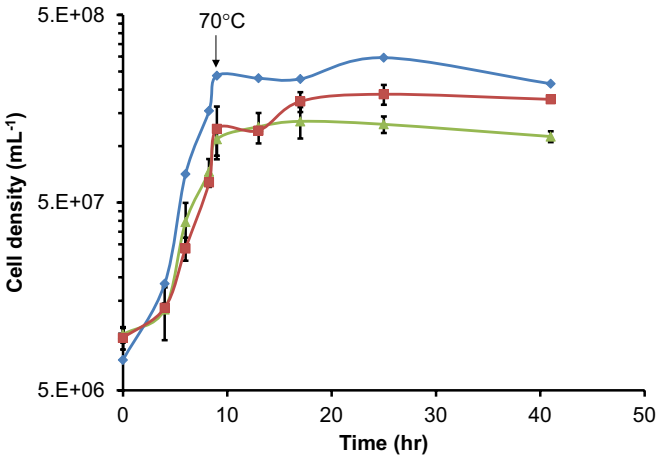

Fig. S6. Growth of *P. furiosus* COM1, MW56, and PF506 during the temperature shift from 98°C to 70°C. Cell densities of COM1 (blue diamonds), MW0056 (red squares), and PF506 (green triangles) are indicated. The 400-mL cultures were grown at 95°C for 9 h and then allowed to cool at room temperature to 70°C before being placed in a 70°C incubator.

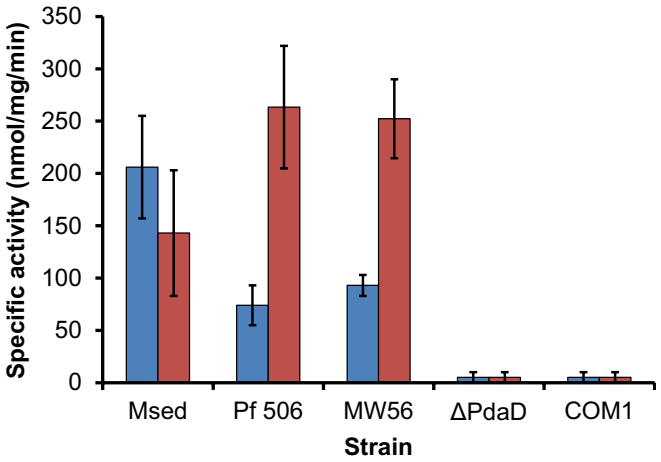

Fig. S7. Enzyme activities of E1 (blue) and coupled E2 + E3 (red) in cell-free extracts of the indicated *P. furiosus* strains after incubation at 70°C for 16 h compared with that measured for the cell extract of autotrophically grown *Metallosphaera sedula* cells (labeled Msed).

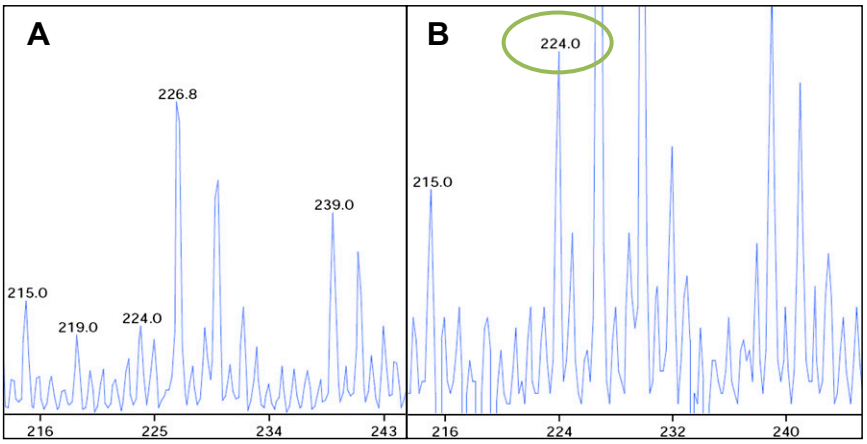

Fig. S8. Electrospray ionization mass spectrometry identification of 3-hydroxypropionic acid produced from acetyl coenzyme A (acetyl-CoA), CO_2, and H_2 (or NADPH) by cell-free extracts of *P. furiosus* strains ΔpdaD (*A*) and PF506 (*B*). The MS peak corresponding to the 3-HP derivative (*m/z* 224, green circle) was present above background only in the recombinant PF506 strain.

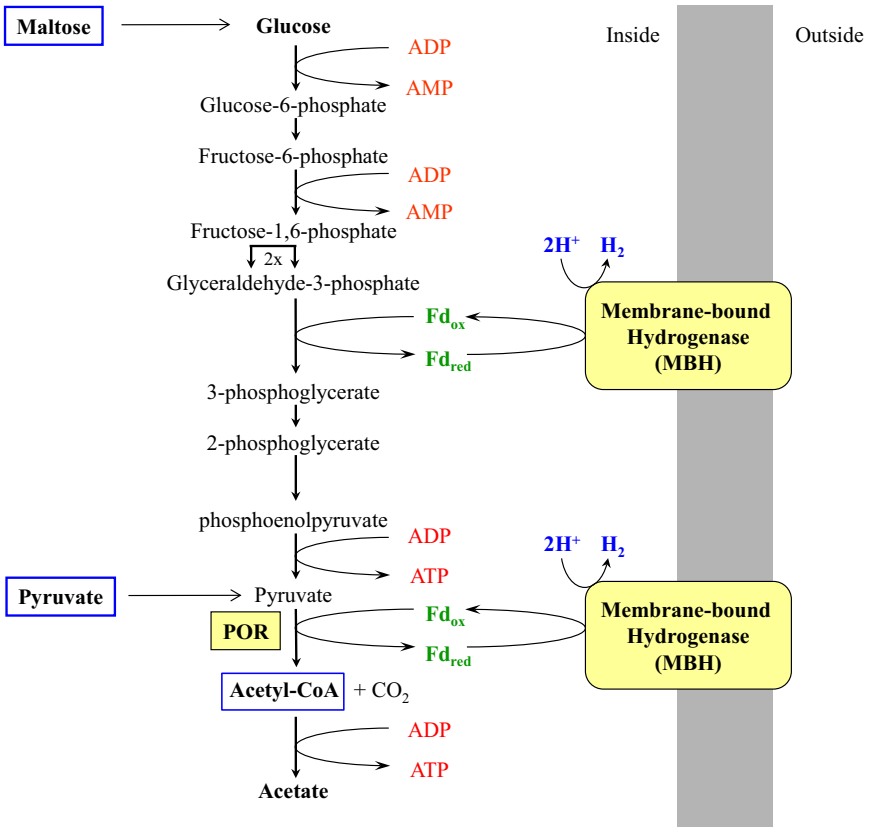

Fig. S9. Maltose and pyruvate metabolism by *P. furiosus* and the key roles of pyruvate ferredoxin oxidoreductase (POR) in acetyl-CoA production and of the membrane-bound hydrogenase in H_2 production.

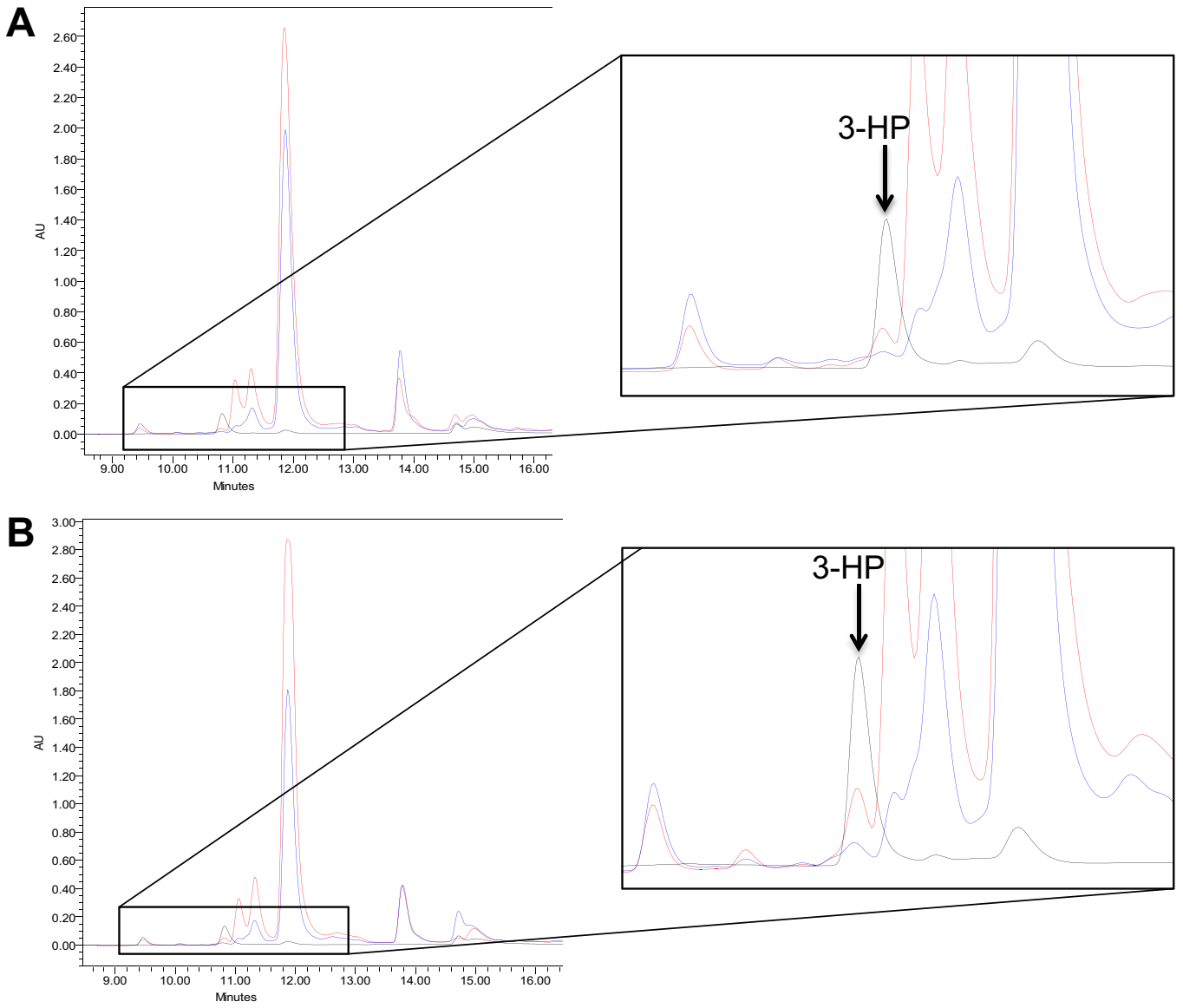

Fig. S10. In vivo production of 3-hydroxypropionic acid (3-HP) from maltose by whole cells of *P. furiosus* strain MW56 (*A*) and PF506 (*B*) after 10 min (blue) and 60 min (red) compared with a 1-mM 3-HP standard (black). A black arrow indicates the position of the 3-HP peaks. A total of 135 μM and 199 μM 3-HP was produced by cell suspensions of MW56 (5×10^{10} cells/mL) and PF506 (5×10^{10} cells/mL), respectively, after 60 min at 75°C.

Table S1. Strains used and constructed in this study

Strain	Parent	Genotype/description	Source
COM1	DSM 3638	Δ*pyrF*	(1)
ΔpdaD	COM1	Δ*pyrF* Δ*pdaD*::P_{gdh}*pyrF*	(2)
PF506	ΔpdaD	Δ*pyrF* Δ*pdaD*::*pdaD* P_{slp}^{-}E1αβγ-E2-E3	This work
MW56	COM1	Δ*pyrF* P_{gdh}*pyrF* P_{slp}^{-}E1αβγ-E2-E3	This work

E1αβγ, acetyl/propionyl-CoA carboxylase; E2, malonyl/succinyl-CoA reductase; E3, malonate semialdehyde reductase; *gdh*, glutamate dehydrogenase; *pdaD*, arginine decarboxylase; P_{slp}, *P. furiosus* S-layer gene promoter; *pyrF*, orotidine-5′-phosphate decarboxylase.

1. Lipscomb GL, et al. (2011) Natural competence in the hyperthermophilic archaeon Pyrococcus furiosus facilitates genetic manipulation: Construction of markerless deletions of genes encoding the two cytoplasmic hydrogenases. *Appl Environ Microbiol* 77(7):2232–2238.
2. Hopkins RC, et al. (2011) Homologous expression of a subcomplex of *Pyrococcus furiosus* hydrogenase that interacts with pyruvate ferredoxin oxidoreductase. *PLoS ONE* 6(10):e26569.

Table S2. Gas chromatography–mass spectrometry identification and quantitation of 3-hydroxypropionic acid produced from malonyl-CoA and NADPH or H_2 by cell-free extracts of *P. furiosus* strain PF506

Vial	Added electron donor	Substrate	Theoretical 3-HP, mM	3-HP/inositol peak area	Estimated 3-hydroxypropionic acid, mM
1	2 mM NADPH	2 mM malonyl-CoA	1	0.0288	0.2
2	2 mM NADPH, H_2	2 mM malonyl-CoA	2	0.0467	0.3
3	1 mM NADP, H_2	2 mM malonyl-CoA	2	0.0274	0.2
4	1 mM NADP, H_2	None (control)	0	0.0064	0.05
5	1 mM NADP, H_2	None (control)	2	0.2839	2.0

The assays were carried out in a total volume of 1 mL containing 0.25 mg cell-free extract under H_2 in a shaking water bath. The amount of 3-hydroxypropionic acid produced was determined after 2 h at 72°C.

Table S3. 3-Hydroxypropionic acid production by whole cells using maltose or pyruvate as the source of acetyl-CoA

P. furiosus strain	Pyruvate	Maltose
MW56	155 nmol	100 nmol
PF506	70 nmol	145 nmol

The amount of 3-hydroxypropionic acid indicated was present in 1 mL of the *P. furiosus* cell suspension.

Table S4. Primers used in the construction of the synthetic subpathway 1 operon

Primer target	Direction	5′ to 3′ sequence
P. furiosus S-layer promoter	Forward	GAATCCCCGCGGCCCGGGCTGGCAGAATAGAA
	Reverse	GCAACCAAAACTCTACTAAAGGGTGGCATTTTTCTCCACCTCCCAATAATCTG
Msed_0147-0148	Forward	ATGCCACCCTTTAGTAGAGTTTTGG
	Reverse	GTTGCAGTCATCTTCAAACCTCCTTACTTTATCACCACTAGGATATCTCC
Msed1375	Forward	GTGATAAAGTAAGGAGGTTTGAAGATGACTGCAACTTTTGAAAAACCGGAT
	Reverse	CGTTCTCCTCATATGCTCCACCTCCCTTAGAGGGGTATATTTCCATGCTTC
Msed_0709	Forward	GGCAATGTCATATGAGGAGAACGCTAAAGGCCGCAATTC
	Reverse	CCTTTTCAGTCATTGCATATCACCTCATCTCTTGTCTATGTAGCCCTTC
Msed_1993	Forward	TAGACAAGAGATGAGGTGATATGCAATGACTGAAAAGGTATCTGTAGTTGGAG
	Reverse	CCAATGCATGCTTATTTTTCCCAAACTAGTTTGTATACCTTC