Supplementary Information for

^{2}H/^{1}H variation in microbial lipids is controlled by NADPH metabolism

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Supporting Information Text

Model description

The overall topology of our model is shown in Figure S1. Isotopic compositions ($^2H/^1H$ ratios) are represented by R's, and molar fluxes by J's. Two distinct cases need to be considered separately, that of under or balanced production of NADPH, and that of over production. In the former case, the membrane-bound transhydrogenase (J_U) is active, while the soluble transhydrogenase is not ($J_O = 0$). Conversely, in the latter case the soluble transhydrogenase (J_O) is active while the membrane-bound transhydrogenase is not ($J_U = 0$). $R_{acetate}$, $J_{acetate}$, R_{water}, and J_{water} are treated as constants in the model (see discussion in main text).

When NADPH is underproduced, there are 5 sources of NADPH (glucose-6P-dehydrogenase, J_G; 6P-gluconate dehydrogenase, J_P; isocitrate dehydrogenase, J_I; malic enzyme, J_M; and membrane-bound transhydrogenase, J_U) and only one sink (J_{NADPH}). During balanced production, $J_U = 0$ and there are only 4 sources. Regardless, to satisfy mass balance, the flux and isotopic composition of the output of NADPH must equal the sum of the inputs, thus:

$$J_{\mathrm{NADPH}} = J_{\mathrm{G}} + J_{\mathrm{P}} + J_{\mathrm{I}} + J_{\mathrm{M}} + J_{\mathrm{U}} \quad [1]$$

$$J_{\mathrm{NADPH}} R_{\mathrm{NADPH}} = J_{\mathrm{G}} R_{\mathrm{G}} + J_{\mathrm{P}} R_{\mathrm{P}} + J_{\mathrm{I}} R_{\mathrm{I}} + J_{\mathrm{M}} R_{\mathrm{M}} + J_{\mathrm{U}} R_{\mathrm{U}} \quad [2]$$

The four dehydrogenase fluxes are measured. J_U is calculated based on the anabolic NADPH demand which is estimated from the measured biomass yield. All five input isotopic compositions are fit by the model.

When NADPH is overproduced, there are now four sources (J_G, J_P, J_I, J_M) and two sinks (J_O and J_{NADPH}). This represents a branchpoint in the reaction network, so the isotopic composition of the sinks of NADPH will depend on i) the sources, ii) the fractionation between the two sinks for NADPH, and iii) the branching ratio of NADPH between anabolism and conversion to NADH (1). For simplicity, we first summarize the four sources in a single term:

$$J_{\mathrm{in}} = J_{\mathrm{G}} + J_{\mathrm{P}} + J_{\mathrm{I}} + J_{\mathrm{M}} \quad [3]$$

$$R_{\mathrm{in}} = \frac{J_{\mathrm{G}} R_{\mathrm{G}} + J_{\mathrm{P}} R_{\mathrm{P}} + J_{\mathrm{I}} R_{\mathrm{I}} + J_{\mathrm{M}} R_{\mathrm{M}}}{J_{\mathrm{in}}} \quad [4]$$

The expression of isotopic mass balance for our system is then

$$J_{\mathrm{in}} = J_{\mathrm{NADPH}} + J_{\mathrm{O}} \quad [5]$$

$$J_{\mathrm{in}} R_{\mathrm{in}} = J_{\mathrm{NADPH}} R_{\mathrm{NADPH}} + J_{\mathrm{O}} R_{\mathrm{O}} \quad [6]$$

The two sinks for NADPH can both be fractionating, leading to different isotopic compositions for R_{NADPH} and R_O. Normally these fractionations would be described by separate α values

$$\alpha_{\mathrm{O/in}} = \frac{R_{\mathrm{O}}}{R_{\mathrm{in}}} \quad [7]$$

$$\alpha_{\mathrm{NADPH/in}} = \frac{R_{\mathrm{NADPH}}}{R_{\mathrm{in}}} \quad [8]$$

However, in this case we cannot resolve the individual fractionations, only the product of the two as expressed in the two sinks for NADPH. For simplicity we thus define a single fractionation factor between the two sinks as

$$\alpha_{\mathrm{O}} = \frac{\alpha_{\mathrm{O/in}}}{\alpha_{\mathrm{NADPH/in}}} = \frac{R_{\mathrm{O}}}{R_{\mathrm{in}}} \frac{R_{\mathrm{in}}}{R_{\mathrm{NADPH}}} = \frac{R_{\mathrm{O}}}{R_{\mathrm{NADPH}}} \quad [9]$$

Substituting Equation 9 into 6 then gives

$$J_{\mathrm{in}} R_{\mathrm{in}} = J_{\mathrm{NADPH}} R_{\mathrm{NADPH}} + J_{\mathrm{O}} (\alpha_{\mathrm{O}} R_{\mathrm{NADPH}}) \quad [10]$$

which can be rearranged to solve for R_{NADPH}

$$R_{\mathrm{NADPH}} = \frac{J_{\mathrm{in}} R_{\mathrm{in}}}{J_{\mathrm{NADPH}} + J_{\mathrm{O}} \alpha_{\mathrm{O}}} \quad [11]$$

Substituting Equation 3 - 5 into Equation 11 gives Equation 3 in the main text

$$R_{\mathrm{NADPH}} = \frac{J_{\mathrm{G}}R_{\mathrm{G}} + J_{\mathrm{P}}R_{\mathrm{P}} + J_{\mathrm{I}}R_{\mathrm{I}} + J_{\mathrm{M}}R_{\mathrm{M}}}{J_{\mathrm{G}} + J_{\mathrm{P}} + J_{\mathrm{I}} + J_{\mathrm{M}} + J_{\mathrm{O}}(\alpha_{\mathrm{O}} - 1)} \quad [12]$$

For both NADPH overproduction and underproduction, the isotope composition of fatty acid (R_{FA}) is calculated from

$$R_{\mathrm{FA}} = \frac{J_{\mathrm{NADPH}}R_{\mathrm{NADPH}} + J_{\mathrm{water}}R_{\mathrm{water}} + J_{\mathrm{acetate}}R_{\mathrm{acetate}}}{J_{\mathrm{NADPH}} + J_{\mathrm{water}} + J_{\mathrm{acetate}}} \quad [13]$$

Based on the known stoichiometry of fatty acid biosynthesis (2, 3), the flux from NADPH has to be twice as high as the flux from water and acetate. Therefore Equation 13 can be simplified as follows:

$$R_{\mathrm{FA}} = 0.5R_{\mathrm{NADPH}} + 0.25R_{\mathrm{water}} + 0.25R_{\mathrm{acetate}} \quad [14]$$

Correlation between NADPH over/underproduction and ^{2}H/^{1}H fractionation in *E. coli* knockout mutants

Strain JW1841 is a G6PDH deletion mutant that catabolizes glucose almost exclusively by the EMP pathway. Because almost no NADPH is generated by G6PDH and 6PGDH (some leakage is observed), JW1841 exhibits a strong underproduction of NADPH. This shortfall is compensated by PntAB (4) and increased ICDH and ME fluxes. Despite these differences, lipid δ^2H values in this mutant are almost equal to those of wildtype fatty acids (Table 1, Figure 3). In strain JW3985, deletion of glucose-6-phosphate isomerase blocks the EMP pathway. This mutation forces glucose metabolism primarily through the PP pathway and led to the largest flux changes that were detected in all investigated mutants (Table 1). Fluxes through ED, PP, and TCA pathways were all considerably increased relative to the wild type *E. coli* and the lipid δ^2H value was over 40‰ higher. With this differences, JW3985 strain falls far off of the correlations for 6PGDH and ICDH (Figure 2), but well within the correlation for the ED pathway. This apparently conflicting result is potentially related to the extreme +164% NADPH imbalance that results from increased PP activity. The overproduction is compensated by the soluble transhydrogenase UdhA (4), which is particularly interesting because it is the only investigated *E. coli* culture that relies on UdhA (4) and yields ^{2}H-enriched lipids relative to all other *E. coli* cultures (Figure 3). ^{2}H/^{1}H fractionation by the soluble transhydrogenase is therefore thought to be the dominant process for generating the lipid δ^2H values in this mutant. In the mutants PntAB and UdhA, the membrane-bound (PntAB) and soluble (UdhA) transhydrogenases are knocked out, respectively. In the UdhA-PntAB double knockout mutant, both transhydrogenases are deleted. In order to still meet the anabolic NADPH demands without the membrane-bound transhydrogenase, the mutants PntAB and UdhA-PntAB raised their PP pathway fluxes which led to balanced NADPH fluxes in the double knockout mutant and a slightly negative NADPH balance in the mutant PntAB (Table 1). Knockout mutant PntAB falls off of the correlation for transhydrogenase fluxes (Figure 3) because it generates more ^{2}H-enriched lipids than the wildtype and all the mutants with negative NADPH balance. In this mutant, the shortfall of NADPH cannot be balanced by the supposedly ^{2}H-depleting PntAB transhydrogenase which may lead to the observed D-enrichment and growth defect (Table S4). In the double knockout mutant UdhA-PntAB, growth is partly restored because NADPH production and consumption is perfectly balanced. Instead of PntAB, the considerably higher PP pathway flux seems to deplete lipids in ^{2}H to a similar extend as PntAB in the wildtype does (Figure 3). In mutant UdhA, carbon fluxes were similar to those in the wild type and NADPH balance is negative as well (Table 1, Figure 2). Because the soluble transhydrogenase is not required when NADPH balance is negative, growth rate, fluxes, and δ^2H values are not significantly affected in the mutant UdhA (Figure 3, Table 1 and S4).

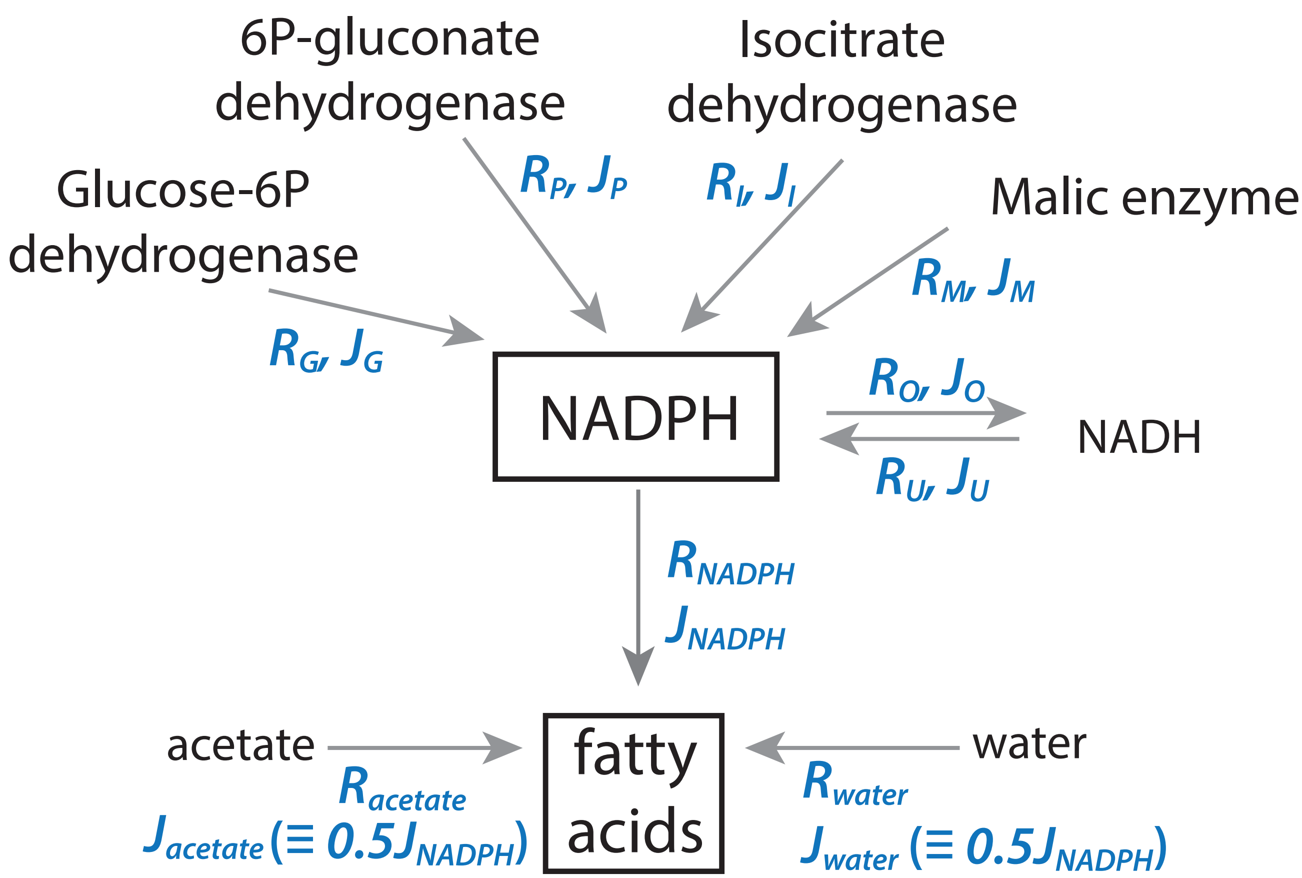

Fig. S1. Overall topology of our model. R's represent $^{2}H/^{1}H$ ratios and J's molar fluxes.

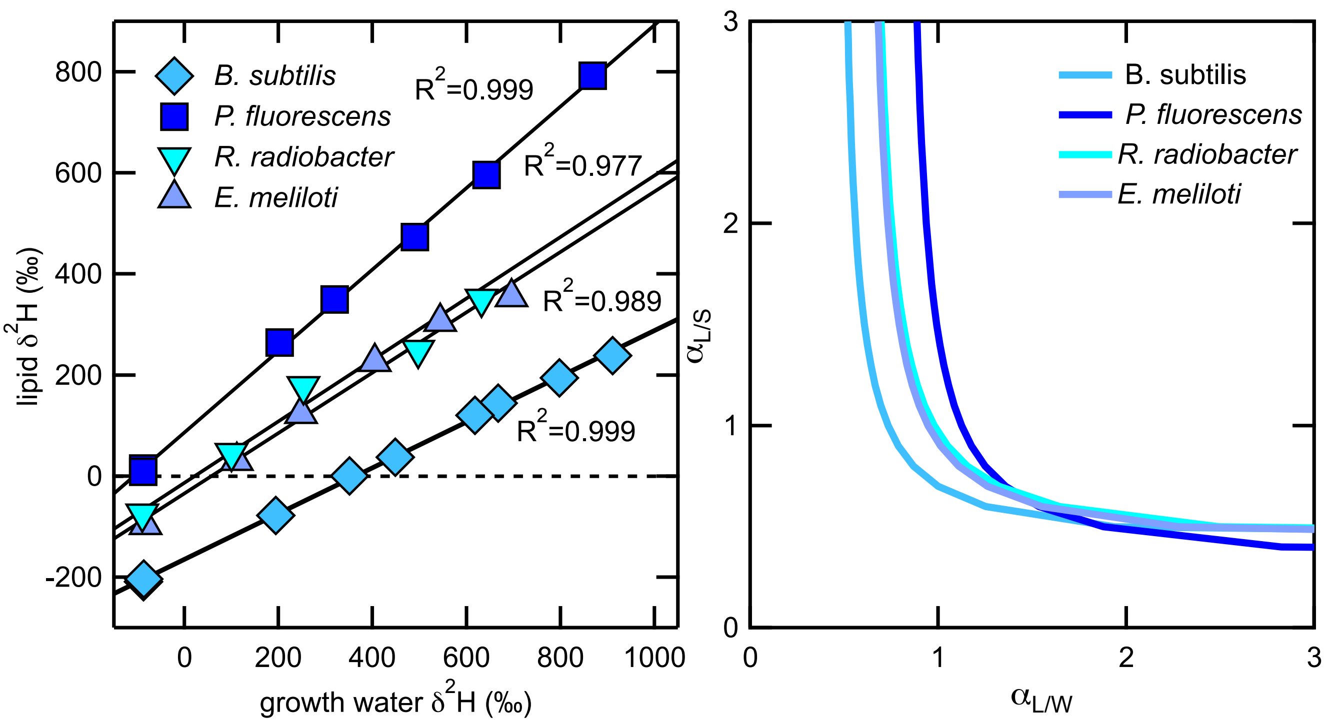

Fig. S2. Correlations between fatty acid δ^2H values and culture medium δ^2H values in *B. subtilis*, *P. fluorescens* WCS365, *R. radiobacter*, and *E. meliloti* (left panel). All cultures were grown on glucose. The same experiment was performed with *E. coli* and other organisms in a previous study (5). In all experiments, lipid δ^2H values are strongly correlated with those of growth medium water (R^2>0.97) with regression slopes ranging from 0.45 to 0.8. A direct interpretation of these correlation slopes as fractional water incorporation (X_w) is not possible because of unknown fractionations between lipids and the two hydrogen sources ($\alpha_{L/W}$, and $\alpha_{L/S}$) (5, 6). However, a comparison of fractionation curves (right panel) following the discussion of Zhang et al. (5) is possible and suggests an increase in $\alpha_{L/W}$ but rather constant X_w and $\alpha_{L/S}$ values in the four strains

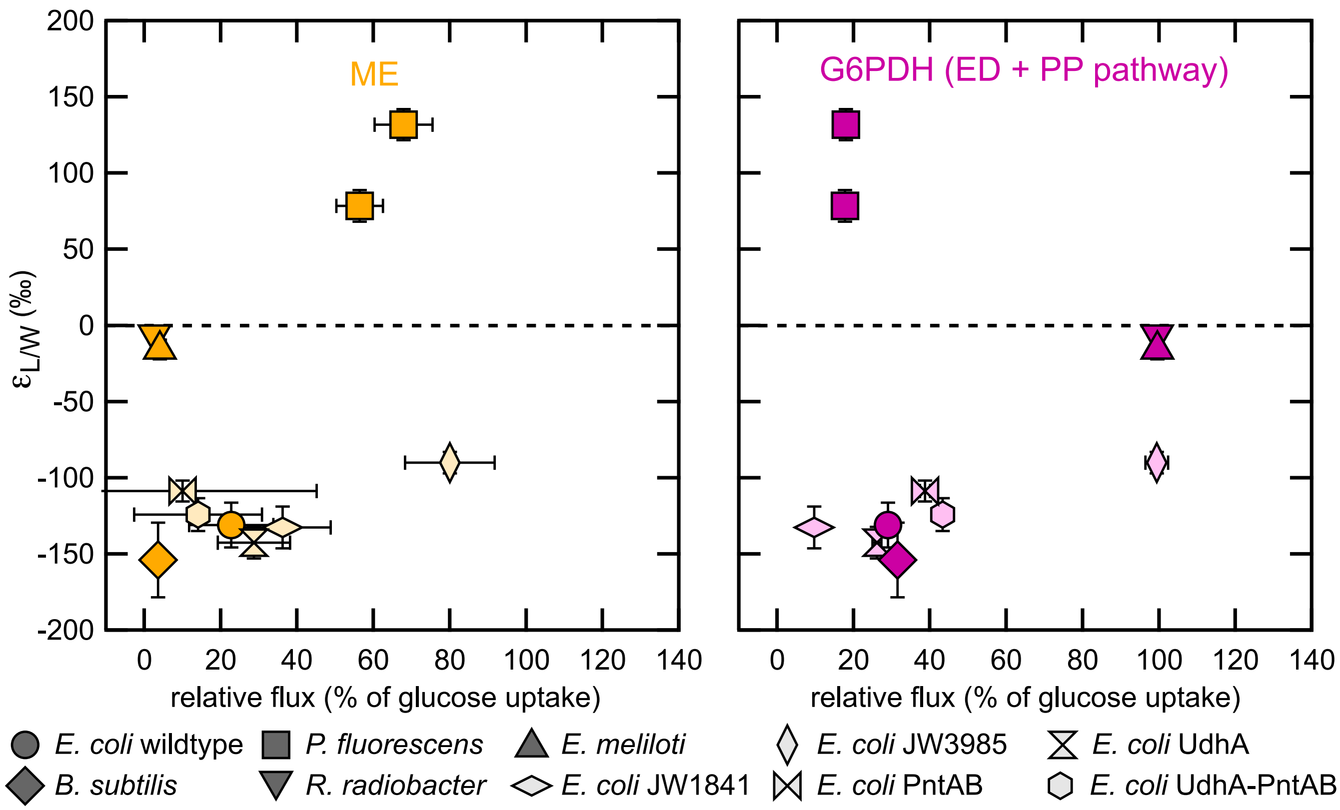

Fig. S3. The relationship between lipid/water fractionation ($\epsilon_{\mathrm{L/W}}$) and relative carbon flux through the NADPH generating reactions catalyzed by ME (left panel) and G6PDH (right panel). Plotted $\epsilon_{\mathrm{L/W}}$ values are calculated between δ^2H values of culture medium and abundance-weighted mean δ^2H values of fatty acids. The error bars represent the corresponding abundance-weighted standard deviation. Note that some error bars are smaller than marker sizes.

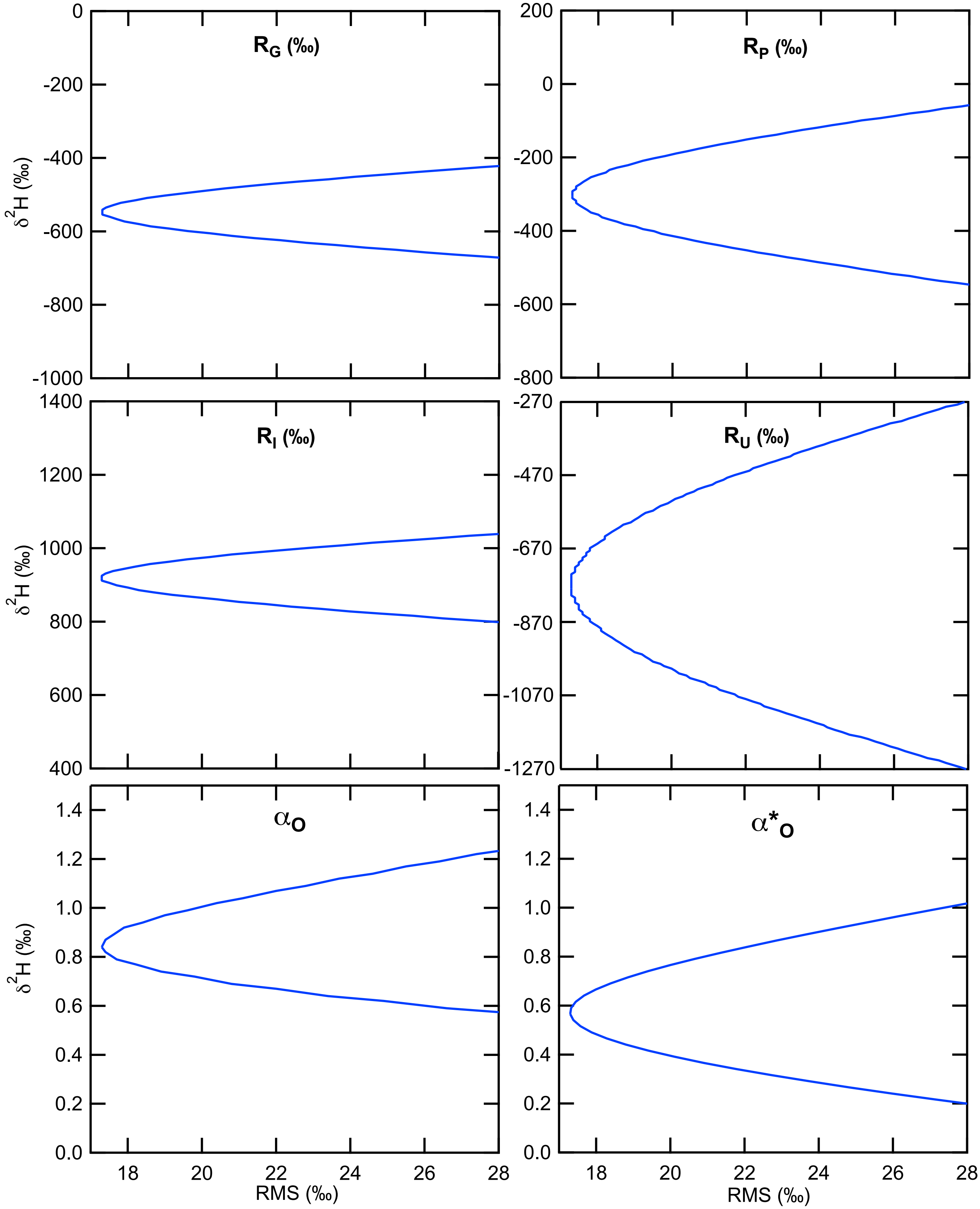

Fig. S4. Sensitivity analysis for the best fit parameters. The relationship between the change in one parameter at a time and the corresponding increase in RMS is shown. R_G account for G6PDH, R_P for 6PGDH, and R_I for ICDH. R_U and α_O account for the membrane-bound (PntAB) and soluble (UdhA) transhydrogenase from *E. coli*, respectively. α^*_O account for the soluble transhydrogenases or alternative mechanisms to balance NADPH levels in all other species. The conclusion that H from 6PGDH and G6PDH is strongly ^{2}H-depleted relative to that from ICDH appears robust, also that both transhydrogenases exhibit normal KIEs.

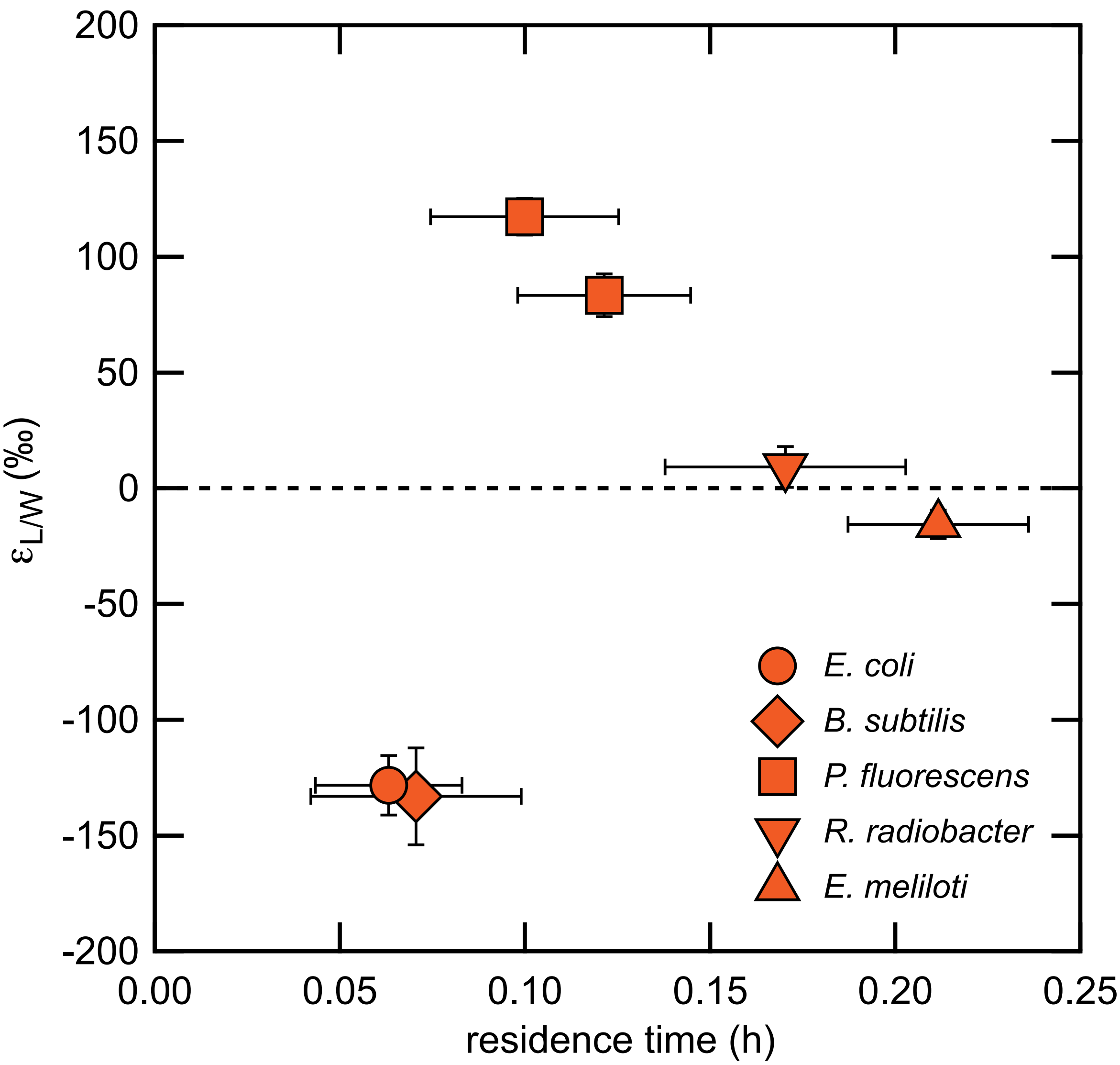

Fig. S5. Lipid/water fractionation ($\epsilon_{\mathrm{L/W}}$) versus NADPH turnover time for the six wildtype strains. Turnover times are calculated from production rates based on metabolic fluxes and intracellular NADPH concentrations.

Table S1. Relative abundances of fatty acids in bacterial cultures.

Nr.	Strain	S†	n‡	Relative abundance of fatty acids* (%)													
				12:0	i14:0	14:0	i15:0	a15:0	15:0	i16:0	16:1	16:0	i17:0	a17:0	cyc17	18:1	19:1
E.coli WT																	
1	MG1655	glu	1	3	-	6	-	-	3	-	21	41	-	-	13	14	-
2		pyr	1	3	-	8	-	-	-	-	13	43	-	-	20	11	1
3		ace	2	3	-	8	-	-	1	-	13	44	-	-	19	9	2
***E.coli* knockout mutants**																	
4	JW1841	glu	1	2	-	4	-	-	-	-	17	32	-	-	15	28	2
5	JW3985	glu	1	2	-	5	-	-	-	-	19	38	-	-	19	17	-
6	PntAB	glu	1	3	-	7	-	-	1	-	22	42	-	-	10	15	-
7	UDHA	glu	1	3	-	7	-	-	-	-	26	40	-	-	8	16	-
8	PntAB UDHA	glu	1	4	-	6	-	-	-	-	24	42	-	-	9	15	-
B.subtilis																	
9	PY79	glu	1	-	2	-	13	45	-	8	-	4	8	21	-	-	-
10		glu	1	-	-	-	19	48	-	9	-		8	16	-	-	-
11		glu	1	-	3	-	22	40	-	9	-	3	10	14	-	-	-
12		glu	1	-	3	-	17	44	-	10	-	5	7	14	-	-	-
13		glu	1	-	3	-	17	45	-	9	-	5	8	14	-	-	-
14		glu	1	-	2	-	23	40	-	8	-	3	11	13	-	-	-
15		glu	1	-	2	-	19	45	-	8	-	3	8	15	-	-	-
16		glu	1	-	3	-	20	41	-	9	-	4	9	14	-	-	-
17		pyr	3	-	3	-	22	41	-	10	-	4	8	11	-	-	-
18		suc	3	-	6	-	17	42	-	19	-	5	-	11	-	-	-
P.fluorescens																	
19	WCS365	glu	1	6	-	-	-	-	-	-	40	40	-	-	-	15	-
20		glu	1	8	-	-	-	-	-	-	43	33	-	-		16	-
21		glu	1	3	-	-	-	-	-	-	39	37	-	-	2	19	-
22		glu	1	5	-	-	-	-	-	-	38	36	-	-	2	19	-
23		glu	1	6	-	-	-	-	-	-	39	35	-	-	1	18	-
24		glu	1	4	-	-	-	-	-	-	38	36	-	-	2	20	-
25		fru	3	3	-	-	-	-	-	-	40	38	-	-	4	16	-
26		gal	3	4	-	-	-	-	-	-	29	40	-	-	12	15	-
27		pyr	3	3	-	-	-	-	-	-	40	36	-	-	-	21	-
28		suc	3	4	-	-	-	-	-	-	40	36	-	-	-	20	-
29		ace	3	4	-	-	-	-	-	-	38	36	-	-	2	20	-
30		cit	3	4	-	-	-	-	-	-	40	36	-	-	-	20	-
31		ben	3	2	-	-	-	-	-	-	42	37	-	-	-	19	-
32	2-79	glu	1	2	-	-	-	-	-	-	32	41	-	-	9	16	-
R. radiobacter																	
33	C58	glu	1	-	-	-	-	-	-		4	9	-	-	1	82	5
34		glu	1	-	-	-	-	-	-	-	-	11	-	-	-	83	5
35		glu	1	-	-	-	-	-	-	-	4	4	-	-	-	86	5
36		glu	1	-	-	-	-	-	-	-	-	11	-	-	-	84	5
37		glu	1	-	-	-	-	-	-	-	4	12	-	-	-	78	6
38		glu	1	-	-	-	-	-	-	-	-	11	-	-	-	83	5
39		fru	3	-	-	-	-	-	-	-	10	10	-	-	-	77	3
40		pyr	3	-	-	-	-	-	-	-	4	10	-	-	-	81	5
41		suc	3	-	-	-	-	-	-	-	-	12	-	-	-	88	-
42		ace	3	-	-	-	-	-	-	-	4	13	-	-	-	59	23
E. meliloti																	
43		glu	1	-	-	-	-	-	-	-	2	8	-	-	1	87	3
44		glu	1	-	-	-	-	-	-	-	-	9	-	-	-	85	6
45		glu	1	-	-	-	-	-	-	-	-	9	-	-	-	85	6
46		glu	1	-	-	-	-	-	-	-	-	9	-	-	-	84	6
47		glu	1	-	-	-	-	-	-	-	-	10	-	-	-	83	8
48		glu	1	-	-	-	-	-	-	-	-	12	-	-	-	75	13
49		fru	3	-	-	-	-	-	-	-	-	10	-	-	-	90	-
50		pyr	3	-	-	-	-	-	-	-	-	7	-	-	-	84	9
51		suc	3	-	-	-	-	-	-	-	-	3	-	-	-	40	57
52		ace	3	-	-	-	-	-	-	-	-	9	-	-	-	79	12

* 12:0, dodecanoic acid; i14:0, 12-methyltridecanoic acid; 14:0, tetradecanoic acid; i15:0, 13-methyltetradecanoic acid ; a15:0, 12-methyltetradecanoic acid; 15:0, pentadecanoic acid; i16:0, 14-methylpentadecanoic acid; 16:1, hexadecenoic acid; 16:0, hexadecanoic acid; i17:0, 15-methylhexadecanoic acid; a17:0, 14-methylhexadecanoic acid ; cyc17, cis-9,10-methylene-hexadecanoic acid; 18:1, octadecenoic acid; 19:1, nonadecenoic acid; Relative abundances are calculated from the peak areas of the GC/IRMS chromatograms; † Substrates (S) for culture experiment were glucose (glu), fructose (fru), galactose (gal), pyruvate (pyr), succinate (suc), acetate (ace), citrate (cit), and benzoic acid (ben); ‡ number of replicated batch cultures.

Table S2. Measured δ^2H values of fatty acids and culture media water as well as calculated abundance-weighted mean δ^2H values for each culture.

Nr. *	Fatty acid δ^2H (‰)†														mean δ^2H	
	12:0	i14:0	14:0	i15:0	a15:0	15:0	i16:0	16:1	16:0	i17:0	a17:0	cyc17	18:1	19:1	H_2O	FA
***E.coli* WT**																
1	−206	-	−203	-	-	−230	-	−216	−205	-	-	−195	−204	-	−88	−208
2	−163	-	−156	-	-	-	-	−160	−143	-	-	−133	−132	−119	−91	−143
3	−53	-	−43	-	-	−73	-	−44	−25	-	-	−17	−17	−2	−91	−28
***E.coli* knockout mutants**																
4	−191	-	−193	-	-		-	−223	−201	-	-	−191	−214	−165	−84	−206
5	−168	-	−169	-	-		-	−175	−171	-	-	−160	−158	-	−85	−167
6	−178	-	−178	-	-	−191	-	−183	−171	-	-	−163	−175	-	−74	-174
7	−223	-	−223	-	-		-	−231	−218	-	-	−196	−216	-	−90	−219
8	−204	-	−200	-	-		-	−216	−201	-	-	−180	−201	-	−90	−203
B.subtilis																
9	-	−164	-	−191	−232	-	−171	-	−194	−177	−215	-	-	-	−68	−211
10	-		-	−77	−92	-	−48	-		−63	−62	-	-	-	194	−78
11	-	5	-	14	−16	-	23	-	−13	19	4	-	-	-	351	1
12	-	47	-	42	29	-	55	-	19	49	46	-	-	-	448	37
13	-	132	-	120	111	-	143	-	119	131	126	-	-	-	618	120
14	-	137	-	157	125	-	164	-	115	163	164	-	-	-	667	144
15	-	195	-	201	188	-	213	-	169	207	195	-	-	-	798	195
16	-	216	-	253	223	-	247	-	209	252	262	-	-	-	911	239
17	-	−58	-	−63	−84	-	−47	-	−77	−64	−51	-	-	-	−86	-69
18	-	19	-	−20	−31	-	27	-	3	-	−10	-	-	-	−88	−11
P.fluorescens																
19	26	-	-	-	-	-	-	44	32	-	-		57	-	−81	40
20	268	-	-	-	-	-	-	260	268	-	-	–	266	-	203	264
21	321	-	-	-	-	-	-	348	351	-	-	339	356	-	319	350
22	452	-	-	-	-	-	-	466	482	-	-	457	477	-	490	473
23	578	-	-	-	-	-	-	590	602	-	-	578	608	-	643	596
24	765	-	-	-	-	-	-	786	803	-	-	763	794	-	868	792
25	−32	-	-	-	-	-	-	−36	−30	-	-	−29	−30	-	−87	-32
26	−62	-	-	-	-	-	-	−51	−50	-	-	−33	−39	-	−87	-47
27	90	-	-	-	-	-	-	115	110	-	-	-	120	-	−87	113
28	198	-	-	-	-	-	-	232	219	-	-	-	235	-	−86	227
29	187	-	-	-	-	-	-	272	240	-	-	280	284	-	−87	260
30	82	-	-	-	-	-	-	108	98	-	-	-	116	-	−88	105
31	130	-	-	-	-	-	-	167	156	-	-	-	179	-	−89	165
32	−3	-	-	-	-	-	-	−20	−6	-	-	−4	−1	-	−82	−10
R.radiobacter																
33	-	-	-	-	-	-	-	−98	−81	-	-	−67	−88	−77	−82	−87
34	-	-	-	-	-	-	-	28	74	-	-		41	53	100	45
35	-	-	-	-	-	-	-	158	227	-	-		176	186	253	178
36	-	-	-	-	-	-	-	212	297	-	-		243	250	497	250
37	-	-	-	-	-	-	-	315	410	-	-		344	350	632	351
38	-	-	-	-	-	-	-	418	504	-	-		446	452	714	453
39	-	-	-	-	-	-	-	−99	−68	-	-	-	−90	−75	−88	-87
40	-	-	-	-	-	-	-	44	84	-	-	-	42	48	−87	47
41	-	-	-	-	-	-	-	-	69	-	-	-	35		−87	39
42	-	-	-	-	-	-	-	3	24	-	-	-	−6	1	−89	0
E.meliloti																
43	-	-	-	-	-	-	-	−98	−73	-	-	−86	−92	−78	−76	−90
44	-	-	-	-	-	-	-	−1	56	-	-		28	33	111	30
45	-	-	-	-	-	-	-	73	151	-	-		121	113	248	123
46	-	-	-	-	-	-	-	172	264	-	-		224	203	405	226
47	-	-	-	-	-	-	-	260	348	-	-		304	274	544	306
48	-	-	-	-	-	-	-	321	407	-	-		354	306	696	355
49	-	-	-	-	-	-	-	-	−103	-	-	-	−113	-	−85	-112
50	-	-	-	-	-	-	-	-	3	-	-	-	−15	3	−77	-12
51	-	-	-	-	-	-	-	-	20	-	-	-	6	24	−83	13
52	-	-	-	-	-	-	-	-	18	-	-	-	1	20	−76	5

*The same number (Nr.) in Table S1 lists strain name, carbon source, and fatty acid abundance; †Triplicate measurements were done for each fatty acid. Typical analytical uncertainties are ±3‰ ($\pm\sigma$), replicate precision is ±8‰ ($\pm\sigma$). Fatty acid structures for corresponding abbreviations are listed in Table S1.

Table S3. Estimated net fluxes in mmol g^{-1}h^{-1}

	E. coli MG1655	*B. subtilis* PY79	*E. meliloti*	*R. radiobacter* C58	*P. fluorescens* 2-79	*P. fluorescens* WCS365
GLU + ATP → G6P	8.30 ± 0.10	6.65 ± 0.09	3.64 ± 0.01	3.88 ± 0.02	0.48 ± 0.03	0.67 ± 0.05
GLU → GLO	-*	-	-	-	4.48 ± 0.09	6.25 ± 0.10
GLO + ATP → 6PG	-	-	-	-	3.89 ± 0.09	5.43 ± 0.11
GLO → 2KG → 6PG + NADPH	-	-	-	-	0.58 ± 0.05	0.81 ± 0.06
G6P → 6PG + NADPH	2.40 ± 0.09	2.10 ± 0.07	3.63 ± 0.02	3.86 ± 0.02	0.89 ± 0.06	1.25 ± 0.08
6PG → Ru5P + CO2 + NADPH	1.72 ± 0.11	2.10 ± 0.07	0.37 ± 0.20	0.41 ± 0.18	0.38 ± 0.03	0.49 ± 0.03
G6P → F6P	5.87 ± 0.12	4.36 ± 0.09	0.00 ± 0.02	0.00 ± 0.02	−0.43 ± 0.03	−0.60 ± 0.04
6PG → GAP + PYR	0.69 ± 0.12	0.00 ± 0.00	3.26 ± 0.20	3.45 ± 0.18	4.99 ± 0.10	7.00 ± 0.11
F6P + ATP → 2 GAP	6.68 ± 0.13	5.55 ± 0.08	0.04 ± 0.12	0.06 ± 0.11	−0.35 ± 0.02	−0.48 ± 0.03
X5P + R5P → S7P + GAP	0.55 ± 0.04	0.68 ± 0.02	0.07 ± 0.07	0.09 ± 0.06	0.11 ± 0.01	0.15 ± 0.01
X5P + E4P → F6P + T3P	0.31 ± 0.04	0.51 ± 0.02	0.00 ± 0.06	0.00 ± 0.05	0.00 ± 0.01	0.00 ± 0.01
S7P + T3P → E4P + F6P	0.55 ± 0.04	0.68 ± 0.02	0.07 ± 0.07	0.09 ± 0.06	0.11 ± 0.01	0.15 ± 0.01
GAP → PGA + ATP + NADH	14.29 ± 0.21	11.51 ± 0.17	3.31 ± 0.11	3.52 ± 0.10	4.26 ± 0.09	5.99 ± 0.10
PGA → PEP	13.40 ± 0.22	10.96 ± 0.17	1.46 ± 0.08	1.93 ± 0.08	3.72 ± 0.10	5.36 ± 0.11
PEP → PYR + ATP	9.07 ± 0.76	11.95 ± 0.25	0.63 ± 0.07	1.20 ± 0.07	3.40 ± 0.11	4.99 ± 0.12
PYR → AcCoA + CO2 + NADH	9.61 ± 0.29	8.19 ± 0.16	2.56 ± 0.26	2.89 ± 0.25	6.00 ± 0.23	9.20 ± 0.26
OAA + AcCoA → ICT	2.80 ± 0.33	1.39 ± 0.15	2.01 ± 0.29	2.16 ± 0.29	5.18 ± 0.24	8.23 ± 0.28
ICT → OGA + CO2 + NADPH	2.80 ± 0.33	1.39 ± 0.15	2.01 ± 0.29	2.16 ± 0.29	5.18 ± 0.24	8.23 ± 0.28
OGA → FUM + CO2 + 1.5 ATP + 2 NADH	1.89 ± 0.35	0.90 ± 0.15	1.75 ± 0.32	1.81 ± 0.31	4.62 ± 0.26	7.59 ± 0.30
FUM → MAL	1.89 ± 0.35	0.90 ± 0.15	1.75 ± 0.32	1.81 ± 0.31	4.62 ± 0.26	7.59 ± 0.30
MAL → OAA + NADH	0.00 ± 0.58	0.66 ± 0.06	1.60 ± 0.31	1.71 ± 0.31	1.80 ± 0.10	2.87 ± 0.14
MAL → PYR + CO2 + NADPH	1.89 ± 0.91	0.24 ± 0.12	0.15 ± 0.05	0.11 ± 0.06	2.82 ± 0.20	4.71 ± 0.24
OAA + ATP → PEP + CO2	0.48 ± 0.14	1.35 ± 0.14	0.00 ± 0.01	0.00 ± 0.02	-	-
PEP + CO2 → OAA	4.28 ± 0.85	-	-	-	-	-
PYR + ATP + CO2 → OAA	-	2.82 ± 0.19	0.72 ± 0.11	0.88 ± 0.11	4.99 ± 0.21	7.16 ± 0.24
OAA → PYR + CO2	-	-	-	-	1.02 ± 0.13	1.11 ± 0.18
AcCoA → Acetate + ATP	5.39 ± 0.10	6.19 ± 0.09	-	-	-	-
NADH → NADPH	2.67 ± 0.98	0.72 ± 0.21	-	-	-	-
NADPH → NADH	-	-	1.39 ± 0.36	0.38 ± 0.34	0.42 ± 0.32	2.58 ± 0.38
Respiration	15.42 ± 0.73	11.75 ± 0.10	5.85 ± 0.58	6.31 ± 0.56	12.4 ± 0.51	19.40 ± 0.60

	E. coli knockout mutants JW1841	JW3985	PntAB	UdhA	UdhA-PntAB
GLU + ATP → G6P	7.97 ± 0.10	4.86 ± 0.10	7.10 ± 0.10	10.38 ± 0.10	7.16 ± 0.10
GLU → GLO	-*	-	-	-	-
GLO + ATP → 6PG	-	-	-	-	-
GLO → 2KG → 6PG + NADPH	-	-	-	-	-
G6P → 6PG + NADPH	0.77 ± 0.10	4.83 ± 0.10	2.75 ± 0.08	2.70 ± 0.12	3.10 ± 0.08
6PG → Ru5P + CO2 + NADPH	0.53 ± 0.10	3.05 ± 0.11	2.03 ± 0.09	1.86 ± 0.14	2.52 ± 0.10
G6P → F6P	7.18 ± 0.14	0.02 ± 0.04	4.33 ± 0.1	7.64 ± 0.14	4.03 ± 0.09
6PG → GAP + PYR	0.24 ± 0.13	1.78 ± 0.09	0.71 ± 0.1	0.84 ± 0.16	0.58 ± 0.10
F6P + ATP → 2 GAP	7.33 ± 0.15	1.92 ± 0.07	5.42 ± 0.11	8.53 ± 0.15	5.46 ± 0.11
X5P + R5P → S7P + GAP	0.17 ± 0.03	1.01 ± 0.03	0.66 ± 0.03	0.60 ± 0.05	0.83 ± 0.03
X5P + E4P → F6P + T3P	0.01 ± 0.04	0.90 ± 0.04	0.45 ± 0.03	0.34 ± 0.05	0.63 ± 0.03
S7P + T3P → E4P + F6P	0.17 ± 0.03	1.01 ± 0.04	0.66 ± 0.03	0.60 ± 0.05	0.82 ± 0.03
GAP → PGA + ATP + NADH	14.88 ± 0.23	6.49 ± 0.15	11.96 ± 0.21	18.16 ± 0.23	12.10 ± 0.20
PGA → PEP	14.31 ± 0.24	6.11 ± 0.16	11.10 ± 0.21	17.20 ± 0.24	11.40 ± 0.21
PEP → PYR + ATP	9.81 ± 0.86	1.18 ± 0.45	7.71 ± 2.32	11.48 ± 0.85	8.44 ± 1.04
PYR → AcCoA + CO2 + NADH	11.57 ± 0.30	5.96 ± 0.30	6.53 ± 0.30	12.99 ± 0.29	8.36 ± 0.29
OAA + AcCoA → ICT	3.50 ± 0.34	4.28 ± 0.34	1.86 ± 0.34	4.02 ± 0.33	1.75 ± 0.33
ICT → OGA + CO2 + NADPH	3.50 ± 0.34	4.28 ± 0.34	1.86 ± 0.34	4.02 ± 0.33	1.75 ± 0.33
OGA → FUM + CO2 + 1.5 ATP + 2 NADH	2.88 ± 0.36	3.88 ± 0.36	0.71 ± 0.36	2.98 ± 0.36	1.01 ± 0.36
FUM → MAL	2.88 ± 0.36	3.88 ± 0.36	0.71 ± 0.36	2.98 ± 0.36	1.01 ± 0.36
MAL → OAA + NADH	0.00 ± 0.68	0.00 ± 0.27	0.00 ± 2.14	0.00 ± 0.67	0.00 ± 0.85
MAL → PYR + CO2 + NADPH	2.88 ± 1.00	3.88 ± 0.56	0.71 ± 2.49	2.98 ± 0.98	1.01 ± 1.19
OAA + ATP → PEP + CO2	2.48 ± 0.20	0.19 ± 0.07	0.67 ± 0.12	1.00 ± 0.19	0.53 ± 0.12
PEP + CO2 → OAA	6.64 ± 0.95	4.90 ± 0.50	3.59 ± 2.44	6.15 ± 0.93	3.08 ± 1.14
PYR + ATP + CO2 → OAA	-	-	-	-	-
OAA → PYR + CO2	-	-	-	-	-
AcCoA → Acetate + ATP	7.16 ± 0.10	1.09 ± 0.10	3.15 ± 0.10	7.40 ± 0.10	5.50 ± 0.10
NADH → NADPH	1.21 ± 1.07	-	0.54 ± 2.52	1.44 ± 1.06	-
NADPH → NADH	-	7.97 ± 0.67	-	-	0.02 ± 1.25
Respiration	17.92 ± 0.75	12.3 ± 0.72	10.77 ± 0.73	20.71 ± 0.73	12.21 ± 0.73

* - indicates an absent reaction

Table S4. Physiological parameter and selected metabolic fluxes for the five wildtype species and five *E.coli* knockout mutants all grown on glucose.

Culture	GR*	GU	AS	absolute flux†						
				G6PDH (ED+PP)	6PGDH (PP)	ICDH (TCA)	ME	EMP§	ED	NADPH balance‡
	(h^{-1})	(mmol $g^{-1}h^{-1}$)					(mmol $g^{-1}h^{-1}$)			
Wildtypes										
E. coli	0.57 ± 0.01	8.4 ± 0.5	5.4 ± 0.5	2.4 ± 0.1	1.7 ± 0.1	2.8 ± 0.3	1.9 ± 0.9	5.9 ± 0.1	0.7 ± 0.1	-2.7
B. subtilis	0.38 ± 0.01	6.6 ± 0.5	6.2 ± 0.8	2.1 ± 0.1	2.1 ± 0.1	1.4 ± 0.1	0.2 ± 0.1	4.4 ± 0.1	0.0 ± 0.0	-0.7
P. fluorescens	0.31 ± 0.01	5.0 ± 0.4	0.0 ± 0.0	0.9 ± 0.1¶	0.4 ± 0.0	5.2 ± 0.2	2.8 ± 0.2	-0.4 ± 0.0	5.0 ± 0.1	0.4
P. fluorescens	0.38 ± 0.01	7.0 ± 0.7	0.0 ± 0.0	1.2 ± 0.1¶	0.5 ± 0.0	8.2 ± 0.3	4.7 ± 0.2	-0.6 ± 0.0	7.0 ± 0.1	2.6
R. radiobacter	0.30 ± 0.00	3.9 ± 0.3	0.0 ± 0.0	3.9 ± 0.0	0.4 ± 0.2	2.2 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	3.5 ± 0.2	0.4
E. meliloti	0.17 ± 0.00	3.7 ± 0.4	0.0 ± 0.0	3.6 ± 0.0	0.4 ± 0.2	2.0 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	3.3 ± 0.2	1.4
E.coli knockout mutants‖										
JW1841	0.34 ± 0.01	7.1 ± 0.4	8.1 ± 0.3	0.8 ± 0.1	0.5 ± 0.1	3.5 ± 0.3	2.9 ± 1.0	7.2 ± 0.1	0.2 ± 0.1	-1.2
JW3985	0.24 ± 0.01	4.9 ± 0.1	1.1 ± 0.1	4.8 ± 0.1	3.0 ± 0.1	4.3 ± 0.3	3.9 ± 0.6	0.0 ± 0.0	1.8 ± 0.1	8.0
PntAB	0.33 ± 0.01	8.1 ± 0.3	2.6 ± 0.4	2.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.3	0.7 ± 2.5	4.3 ± 0.1	0.7 ± 0.1	-0.5
UdhA	0.58 ± 0.01	10.6 ± 0.8	7.3 ± 0.9	2.7 ± 0.1	1.9 ± 0.1	4.0 ± 0.3	3.0 ± 1.0	7.6 ± 0.1	0.8 ± 0.2	-1.4
UdhA-PntAB	0.42 ± 0.01	7.3 ± 0.4	5.4 ± 0.4	3.1 ± 0.1	2.5 ± 0.1	1.8 ± 0.3	1.0 ± 1.2	4.0 ± 0.1	0.6 ± 0.1	0

* Growth rate (GR), glucose uptake rate (GU), and acetate secretion rate (AS) were measured. Standard deviations ($\pm\sigma$) are derived from three parallel cultures. † Absolute fluxes are given in mmol $g^{-1}h^{-1}$ for the four NADPH generating reactions (G6PDH, 6PGDH, ICDH, ME) and EMP and ED pathway. ‡ Negative values indicate NADPH underproduction relative to anabolic demand, positive values indicate NADPH overproduction. §Negative fluxes indicate reversed EMP flux. ¶ For *Pseudomonas* species, previously published relative flux distributions (7) were assumed due to the periplasmatic conversion of glucose to gluconate and 2-keto-gluconate. ‖ Mutants carry the following gene deletions: G6PDH in JW1841, glucose-6-phosphate isomerase in JW3985 , membrane-bound transhydrogenase in PntAB , soluble transhydrogenase in UdhA the soluble transhydrogenase, and both transhydrogenae genes in UdhA-PntAB.

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