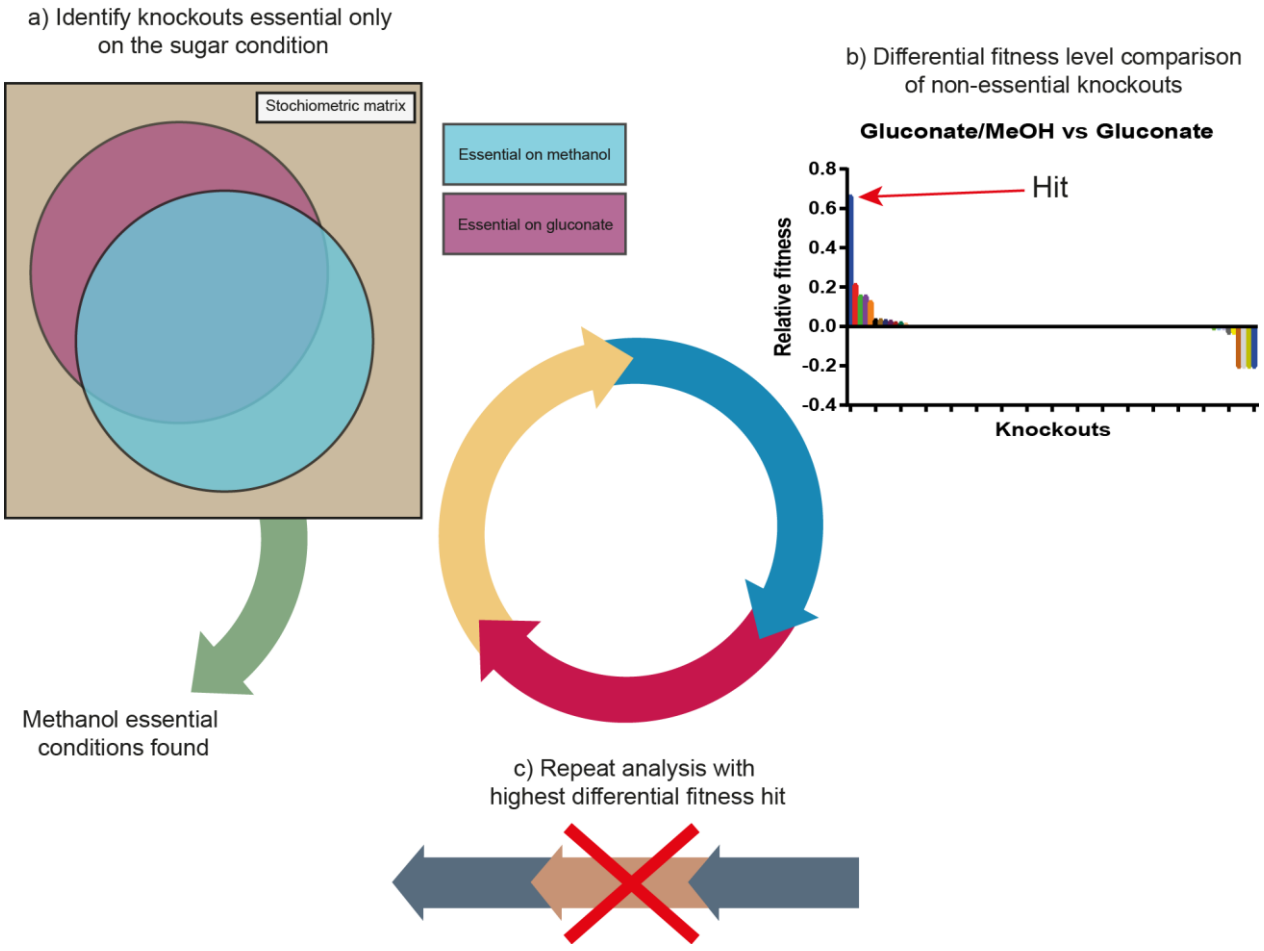
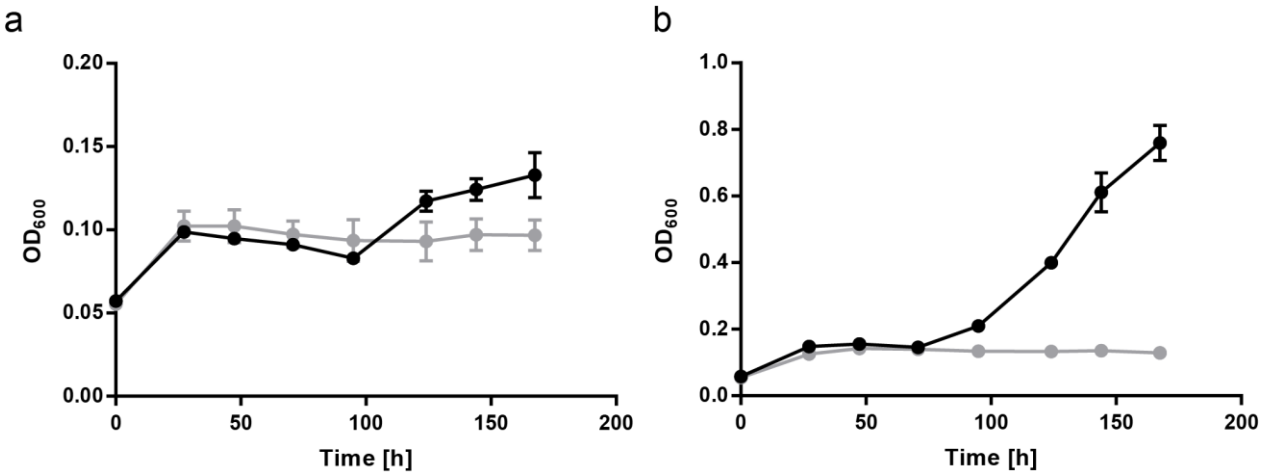
Supplementary Information

Methanol-essential growth of *Escherichia coli*

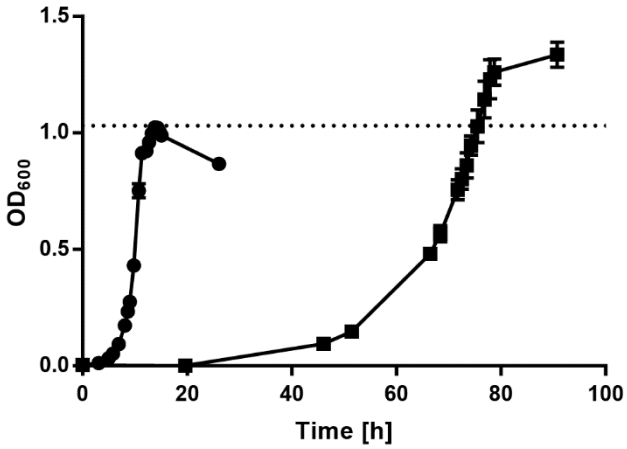
Fabian Meyer, Philipp Keller, Johannes Hartl, Olivier G. Gröninger, Patrick Kiefer, Julia A. Vorholt



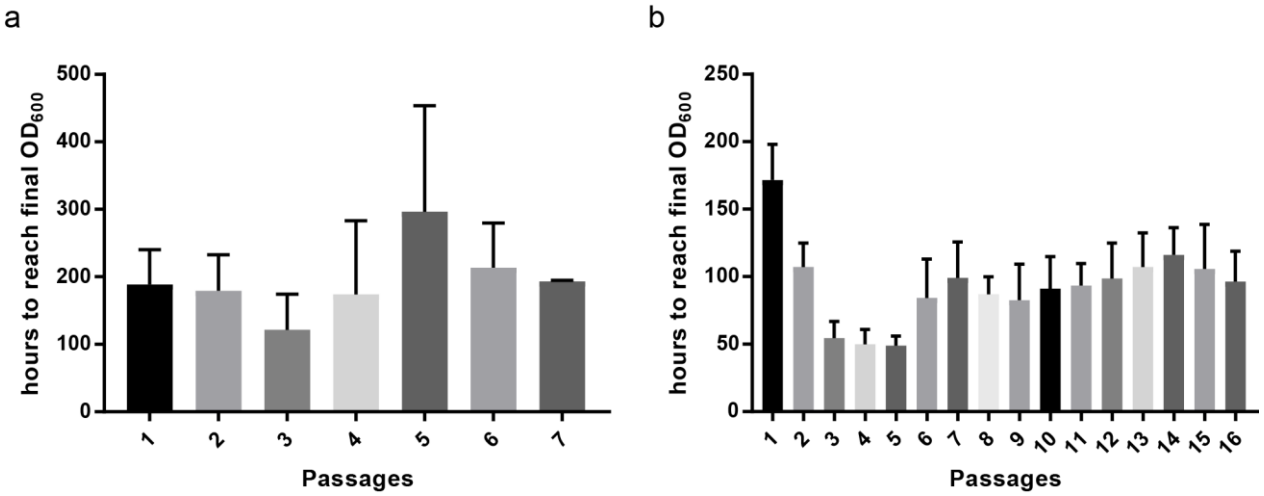
Supplementary Figure 1. Iterative workflow to identify methanol-essential knockouts, gluconate is chosen as case study. a) As a first step, knockouts that are essential only on the sugar condition are identified. b) In case no knockout can be found, differential fitness level comparison of non-essential knockouts is performed comparing predicted growth rates of gluconate vs gluconate/methanol condition. c) Analysis is repeated with highest differential fitness hits until methanol-essential genotype is identified.



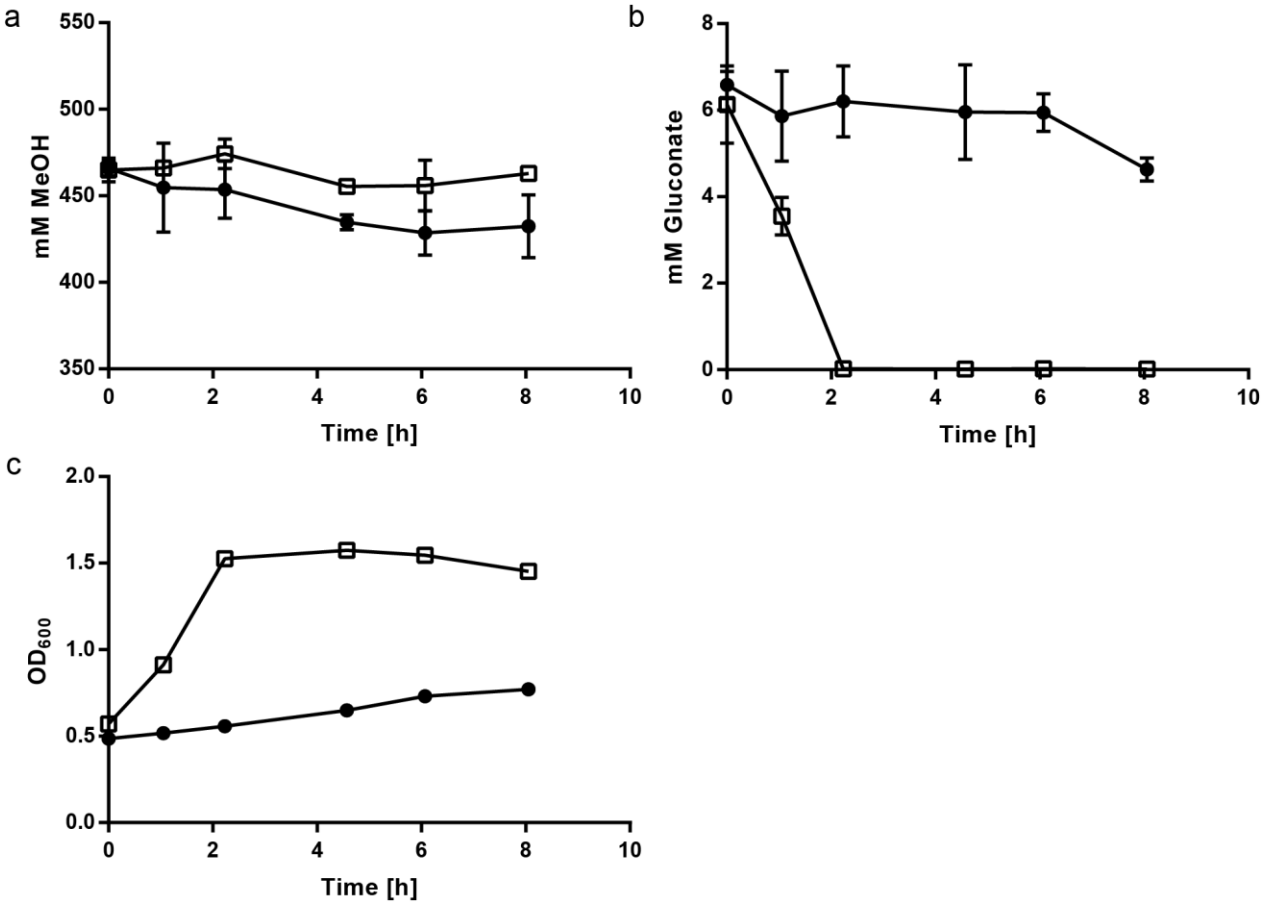
Supplementary Figure 2 Initial growth experiment of methanol-essential strain version 1 (n=3). a) Initial growth experiment on 5 mM gluconate (0.1 g L^{-1} yeast extract) with (black) and without (grey) 500 mM methanol. b) Initial growth experiment on 5 mM gluconate and 20 mM pyruvate (0.1 g L^{-1} yeast extract) with (black) and without (grey) 500 mM methanol. Data presented as mean ± standard deviation.



Supplementary Figure 3 Growth comparison between MeSV2.2 and wild type *E. coli* with empty plasmids. Growth curves of MeSV2.2 (squares) and wild type *E. coli* strain containing empty plasmids (circles) under the same growth conditions; 5 mM gluconate with 500 mM methanol and without yeast extract. Data presented as mean ± standard deviation (n=3).



Supplementary Figure 4 Average time in hours to reach final OD_{600} per passage in case of evolution experiments of MeSV1.1 and MeSV2.1. Data presented as mean ± standard deviation. a) In case of MeSV1.1 (n=5) and b) in case of MeSV2.1 (n=6).



Supplementary Figure 5 Methanol and gluconate consumption of MeSV2.2 (black circles) and wild type *E. coli* containing empty vector control (open squares); n=3. Data presented as mean ± standard deviation. a) Methanol consumption b) gluconate consumption c) growth during consumption.

Supplementary Table 1: Oligonucleotides used in this study.

Oligonucleotides:	**Sequence:**
1_Fwd_Mdh2_*Bacillus_methanolicus*_PB1	GGGGAATTCAGGAGATATACATATGACAAACACTC
2_Rev_Mdh2_ *Bacillus_methanolicus*_PB1	GGGCTGCAGTTACTCGAGCATAGCATTTTTAATAAT
3_Fwd_Hps_Phi_*Methylobacillus_flagellatus*	GGGGAATTCAGGAGAAAGTATCGTGGCATTGAC
4_Rev_Hps_Phi_*Methylobacillus_flagellatus*	GGGAAGCTTTTATTCAAGATTGGCGTGAA
5_rpiA_seq_fw	CGTTGCCCAGCGGCAGTTTATTTTC
6_rpiA_seq_rv	CTACCTGCAAGTTGAGGCGGATAG
7_rpiB_seq_fw	ATCCTCCGGCACCGTACAAATC
8_rpiB_seq_fw	GCAGGTAATGGTGATGGATTGC
9_edd_seq_fw	GCTCCGGTTACAGGCGTTTCAG
10_edd_seq_rv	CACGATAACCGGTACAACCGGG
11_maldh_seq_fw	GGCAGCGGAGCAACATATC
12_maldh_seq_rv	TCTGTGCTCCGGTTTTTTATTATCC
13_pSEVA_seq_fw	CATCCGGCTCGTATAATGTG
14_pSEVA_seq_rv	GAGTTCTGAGGTCATTACTG

Supplementary Table 2: Strains used in this study.

Strain	Genotype	Description	Reference
Wild type empty plasmids	BW25113[3] pSEVA424 pSEVA131	Wild type *E. coli* strain used as control	This study
Wildtype pSEVA424 *mdh2* PB1 pSEVA131 *hps phi* M.f.	BW25113 pSEVA424 *mdh2* PB1 pSEVA131 *hps phi* M.f.	Wild type *E. coli* strain harboring plasmids encoding *mdh2* PB1 and *hpsphi* M.f. used as comparison	This study
MeSV1 (Methanol-essential strain)	BW25113 Δ*rpiA*Δ*rpiB*Δ*edd* pSEVA424 *mdh2* PB1 pSEVA131 *hps phi* M.f.	Methanol dependent strain on gluconate. Parental strain of MeSV1.1	This study
MeSV2 (Methanol-essential strain version 2)	BW25113 Δ*rpiA*Δ*rpiB*Δ*edd*Δ*maldh* pSEVA424 *mdh2* PB1 pSEVA131 *hps phi* M.f.	Methanol dependent strain on gluconate with reduced TCA cycle activity. Parental strain of MeSV2.1 and MeSV2.2	This study

Supplementary Table 3: Plasmids used in this study.

Plasmid	Encoded gene	Description	Reference
pSEVA424		Low-copy-number, lacIq/Ptrc promotor, RK2 ori, Sm^R.	(Silva-Rocha et al., 2013)[1]
pSEVA131		Medium-copy-number, lacIq/Ptrc promotor, pBBR1 ori, Amp^R. Original pSEVA131 plasmid does not contain a promotor.	(Silva-Rocha et al., 2013)[1]
pSEVA424 *mdh2* PB1	*mdh2* gene (PB1_12584) of *B. methanolicus* PB1	*mdh2* gene was amplified from pET21a mdh2 PB1 using primers 1 and 2 and ligated into the *EcoRI*/*PstI* site of pSEVA424	(Müller et al., 2015)[2]
pSEVA131 *hps phi* M.f.	*hps* and *phi* genes (Mfla_1654 and Mfla_1653) of *M. flagellatus* KT (DSM 6875)	*hps phi* operon was amplified from genomic DNA using primers 3 and 4 and ligated into the *EcoRI*/*HindIII* site of pSEVA131	This study

Supplementary References

1. Martínez-Garćía, E., Aparicio, T., Goñi-Moreno, A., Fraile, S. & De Lorenzo, V. SEVA 2.0: An update of the Standard European Vector Architecture for de-/re-construction of bacterial functionalities. *Nucleic Acids Res.* **43,** D1183–D1189 (2015).

2. Müller, J. E. N. *et al.* Engineering *Escherichia coli* for methanol conversion. *Metab. Eng.* **28,** 190–201 (2015).

3. Baba, T. *et al.* Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2,** 2006.0008 (2006)