

Improving methanol assimilation in *Yarrowia lipolytica* via systematic metabolic engineering combined with compartmentalization

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Supplementary Information

Supplemental Table1. Strains and plasmids used in this study.

Strains	Description	Reference
Po1f	Base strain with leu ⁻ and ura ⁻ knockout	Lab collection ³⁹
Y1-001	po1f contained 113-das-dak and pki-aox-cta.	This study
Y1-002	Y1-001 contained 113H- xyr-xdh-xyk.	This study
Y1-003	Mutant of strain Y1-002 that grows using xylose as carbon source.	This study

Y1-004	Y1-003 contained 113H- fbp-fba-tal.	This study
004-Ptp	Y1-004 contained 113H-Ptp.	This study
004-Gnd	Y1-004 contained 113H-Gnd.	This study
004-Hsp70	Y1-004 contained 113H-Hsp70.	This study
004-Tpi	Y1-004 contained 113H-Tpi.	This study
Y1-005	004-Hsp70 knocked out of sdh5	This study
Plasmids		
113	Amp ^R , Clone vector, Uracil, pTef and pGPD	Lab collection
Pki	Amp ^R , Clone vector, Leucine, pUAS1B8-TEF	Lab collection
113H	113 derivative using hygromycin to replace uracil as select maker.	This study
113-das-dak	113 derivative harboring das and dak from <i>P. pastoris</i> .	This study
Pki-aox-cta	113 derivative harboring aox and cta from <i>P. pastoris</i> .	This study

113H-xyr-xdh-xyk	113H derivative harboring xyr, xdh and xyk from <i>M. guilliermondii</i> .	This study
113H- fbp-fba-tal	113H derivative harboring fbp1p, fba2p and tal1p from <i>P. pastoris</i> .	This study
113H-Ptp	113H derivative harboring Ptp from <i>Y. lipolytica</i> .	This study
113H-Gnd	113H derivative harboring Gnd from <i>Y. lipolytica</i> .	This study
113H-Hsp70	113H derivative harboring Hsp70 from <i>Y. lipolytica</i> .	This study
113H-Tpi	113H derivative harboring Tpi from <i>Y. lipolytica</i> .	This study
113H-Shm1-Sda	113H derivative harboring Shm1 and Sda from <i>Y. lipolytica</i> .	This study
pCRISPRyl-shd5	pCRISPRyl derivative harboring sgRNA targeted to sdh5	This study

Supplemental Table 2. Fermentation results of recombination strain from different carbon sources.

Strains	Methanol consumption (g/L)	Cell growth (Max OD ₆₀₀)	Methanol consumption rate (g/L·OD ⁻¹)	Growth Rate (OD/h)	Carbon sources
Y1-001	0.43	0.96	0.448	0.013	10 g/L Methanol+ +1 g/L Yeast extract
Y1-003	3.4	8.7	0.391	0.120	20 g/L Methanol+20 g/L Xylose
Y1-004	4.5	9.1	0.495	0.126	20 g/L Methanol+20 g/L Xylose
004-Ptp	4.7	8.3	0.566	0.115	20 g/L Methanol+20 g/L Xylose
004-Gnd	5.8	9.5	0.611	0.132	20 g/L Methanol+20 g/L Xylose
004-Tpi	5.3	9.3	0.570	0.129	20 g/L Methanol+20 g/L Xylose
004-Hsp70	5.2	10.5	0.495	0.146	20 g/L Methanol+20 g/L Xylose
004-Shm1-Sda	6.2	9.6	0.646	0.133	20 g/L Methanol+20 g/L Xylose

Y1-005 2.8 4.9 0.571 0.068 20 g/L Methanol+20 g/L Xylose

Supplemental Table3. Transcriptome analysis result of relevant genes involved in the methanol metabolism.

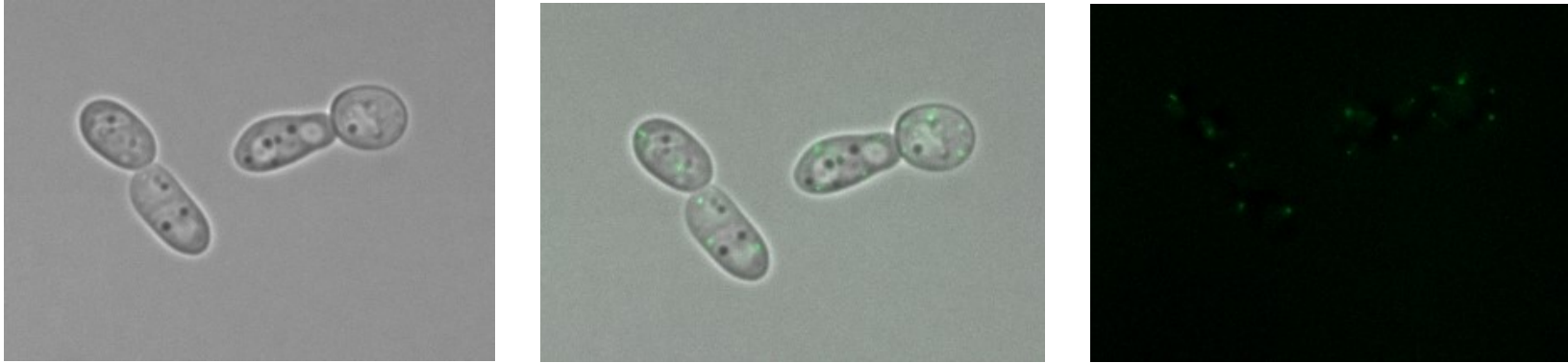
id	baseMean	baseMeanA	baseMeanB	foldChange	log2FoldChange	pval	padj	Description
BKA90DRAFT_97120	727.86594	102.79248	1352.9394	13.161852	3.7182906	0.001615	0.1181513	Tpi
BKA90DRAFT_90876	14428.882	2691.9993	26165.766	9.7198261	3.2809305	0.001873	0.1247406	Sda
BKA90DRAFT_81944	8686.5657	3198.2038	14174.928	4.4321527	2.1480076	0.0338257	0.7249287	AroF
BKA90DRAFT_4102	4144.5337	1525.4016	6763.6658	4.4340229	2.1486162	0.0354603	0.7371152	AroC
BKA90DRAFT_29362	135.77212	25.617373	245.92686	9.600003	3.2630349	0.0398018	0.7884277	Suc
BKA90DRAFT_27005	1209.52	197.82704	2221.213	11.228056	3.4890362	0.0019226	0.1247406	ilvC
BKA90DRAFT_170863	1044.2295	1866.7503	221.70882	0.1187673	-3.073791	0.0058098	0.2661822	Gap
BKA90DRAFT_162970	1459.72	535.2967	2384.1432	4.4538724	2.1550602	0.0410097	0.8076024	Trp3
BKA90DRAFT_157567	7838.7499	1185.9925	14491.507	12.218886	3.6110409	0.0007848	0.0773942	Dak
BKA90DRAFT_145330	39463.108	7680.344	71245.872	9.2763908	3.2135636	0.0021773	0.1357778	Glt1
BKA90DRAFT_143229	2714.9101	528.50852	4901.3117	9.2738557	3.2131693	0.0028137	0.1633648	Got2

BKA90DRAFT_142992	1720.1216	3040.1362	400.10708	0.1316083	-2.925678	0.0066935	0.296582	Gpma
BKA90DRAFT_142835	11512.621	1781.4131	21243.83	11.925269	3.5759499	0.0008347	0.0802583	Cta
BKA90DRAFT_141483	655.01925	91.155597	1218.8829	13.371454	3.7410845	0.0017008	0.1203585	Xyr
BKA90DRAFT_139160	1255.8013	201.706	2309.8965	11.451799	3.5175023	0.0017493	0.1214599	Frm
BKA90DRAFT_136082	491.40273	64.002866	918.8026	14.355648	3.8435465	0.0018819	0.1247406	Mdh
BKA90DRAFT_136038	17909.767	6578.7189	29240.815	4.4447583	2.152105	0.0327359	0.7137357	TrpA
BKA90DRAFT_135217	2324.5005	401.47253	4247.5285	10.579873	3.4032505	0.0017859	0.1227358	Shm1
BKA90DRAFT_134641	5204.7515	967.80092	9441.7021	9.7558309	3.2862648	0.0020328	0.128404	Acl
BKA90DRAFT_133792	2615.1805	470.32409	4760.0368	10.120759	3.3392456	0.00204	0.128404	ilvB
BKA90DRAFT_132755	575.73949	87.276636	1064.2023	12.193439	3.6080332	0.0026521	0.1580684	ilvE
BKA90DRAFT_128453	4342.2316	769.97388	7914.4893	10.278906	3.3616149	0.0017079	0.1203585	Xdh
BKA90DRAFT_11213	10743.196	3966.2382	17520.153	4.4173224	2.1431721	0.0339054	0.7249287	TrpD

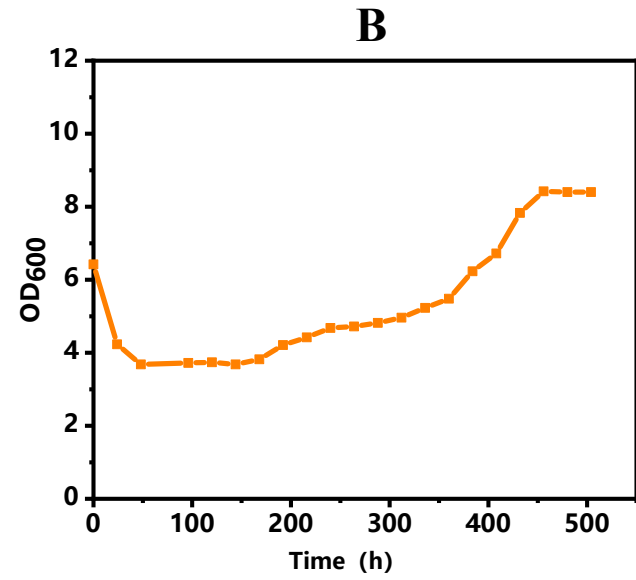
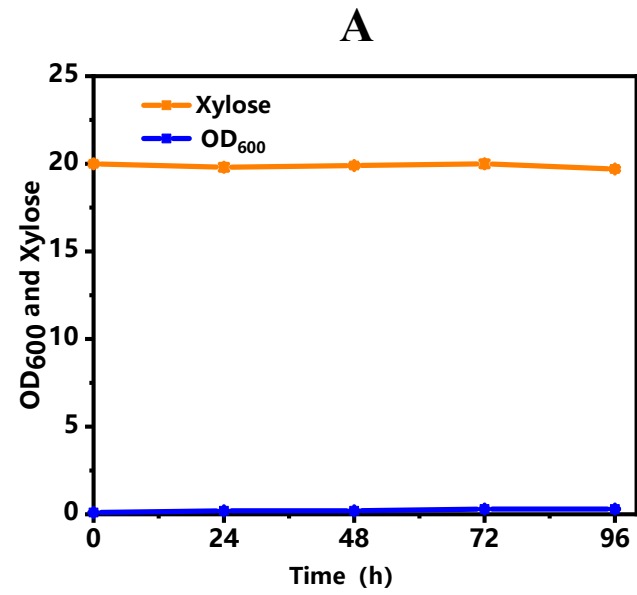
Supplemental Table4. Sequences of primer pairs used in this study. The green section is natural peroxisomal targeting signals and red section is the added peroxisomal targeting signals.

Primer	Sequence	Amplified fragment
das-P1	CAGTACTAACCGCAGATTTAAATATGGCTAGAATTCCAAAAGCAGTATC	Dihydroxyacetone synthase
das -P2	TAACTAATTACATGAATTTAAATTTACAGTTTTGACAACTTGTCATGCTTTGGTTTTTC	
dak-P1	AAACACACATCAACAGTCGACATGTCTAGTAAACATTGGGATTAC	Dihydroxyacetone kinase
dak-P2	AGGCCATGGAGGTACGTCGACCTACAGTTTTGACAACTTGGTTTCAGATTTGAAG	
aox-P1	AGAATCATTCAAAGGTTTCGAAATGGCTATCCCCGAAGAGTTTG	Alcohol oxidase
aox-P2	TAACTAATTACATGATTCGAATTAGAATCTAGCAAGACCGGTCTTC	
cta-P1	CTAGTGGATCCTAGTCATATGTCTGCGTTCGTTAAACCTAAGTAAC	Catalase
cta-P2	AGGCCATGGAGGTACGTCGACCTACAGTTTTGACAATCTTGCTGCAGAGT	
xyr-P1	AAACACACATCAACAGTCGACATGTCTATTACTTTGAACTCAGGT	Xylose reductase
xyr-P2	AGGCCATGGAGGTACGTCGACTTACACAAAAGTTGGAATCTTGTC	
xyk-P1	CAGTACTAACCGCAGATTTAAATATGGAAGCCCTCGTAGACCAGCTC	Xylulokinase
xyk-P2	TAACTAATTACATGAATTTAAATCTAATCATGCTTCAAATTCTCCTC	
x dh-P1	GGAACCCGAAACTAAGGATCCATGTCTTGTAATTTTACTTCAAGCA	Xylitol dehydrogenase
x dh-P2	GGCCATGGAGGTACCGGATCCTTACTCGGGACCATCTATAATAAC	
hph-P1	TTAAACACACATCAACAGTCGACATGCCTGAACTCACCGCGACGTCTG	Hygromycin
hph-P2	ACAGGCCATGGAGGTACGTCGACCTATTCCTTTGCCCTCGGACGAGTG	
fbp-P1	AAACACACATCAACAGTCGACATGTCCAATAACACCACCCAAAAC	Fructose-1,6-bisphosphatase 2

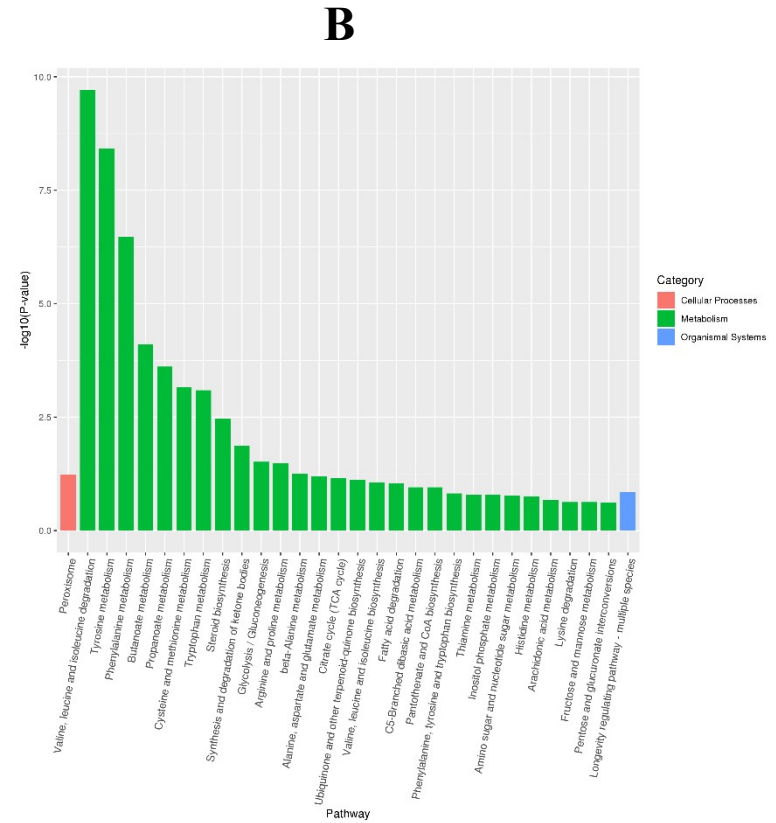
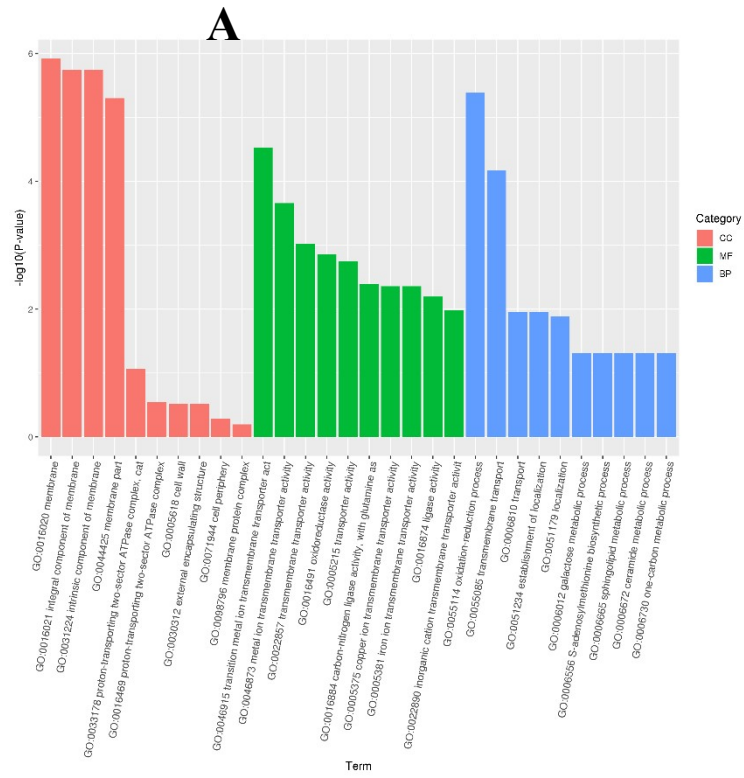
fbp-P2	AGGCCATGGAGGTACGTCGACCTACAGTTTTGAGAGATTCACAGATTGGA TCTTA	
fba-P1	GTACTAACCGCAGATTTAAATATGTCTACATTTGATTTCCCTTTCCAG	Fructose-1,6-bisphosphate aldolase
fba-P2	ACTAATTACATGAATTTAAATTCA CAGTTTGA CT CAGACTTGAAG	
tal-P1	AAACACACATCAACAGTCGACATGGAATCCAATCCTATCAACTC	Transaldolase
tal-P2	AGGCCATGGAGGTACGTCGACTTCAACTTGGATAAAAACACGAC	
Gnd-P1	AACACACATCAACAGTCGACATGACTGACACTTCAAACAT	6-phosphogluconate dehydrogenase
Gnd-P2	GGCCATGGAGGTACGTCGACTTAAGCATCGTAAGTGGAAG	
Tpi-P1	GAATCATTCAAAGGTTGAAATGTCTCGAACCTTTTTTGT	Triose phosphate isomerase
Tpi-P2	AACTAATTACATGATTCGAATTAAGTCGAGAGTTGATGA	
Shm1-P1	TACTAACCGCAGATTTAAATATGCAGCGATTCACTCGACT	serine hydroxymethyltransferase
Shm1-P2	CTAATTACATGAATTTAAATCCCGGCCGCTATTTGTATCCAGCAGGAT	
Ptp-P1	AACACACATCAACAGTCGACATGGCCGCTCTTCACCAATT	phosphate transporter
Ptp-P2	TGGCCATGGAGGTACGTCGACCTAAGACAATTGGTAAACCT	
Sda-P1	AACACACATCAACAGTCGACATGGCCTCCATCATCGACGG	serine deaminase
Sda-P2	GGCCATGGAGGTACGTCGACTTAGAAAAGACCCTCGATCT	
Hsp70-P1	AACACACATCAACAGTCGACATGAGTGAAGGAACTTTTGC	heat shock protein 70
Hsp70-P2	GGCCATGGAGGTACGTCGACTTATCGGGTAGCCATGGCCT	
sgRNA- pme1 F	TTACGCCAAGCTTGTTTGTTTAAACCCCCAGTTGCAAAAGTTGAC	sgRNA-Sdh5
Sdh5-N20 R	CAGGGTTGATGTTGGCCTGGACGTCAACCTGCGCCGACCC	
Sdh5-N20 F	CCAGGCCAACATCAACCCTGGTTTTAGAGCTAGAAATAGC	
sgRNA-sal1 R	GCCAACCCGGTCTCTGTGACAAAAAAAAGCACCGACTCGG	



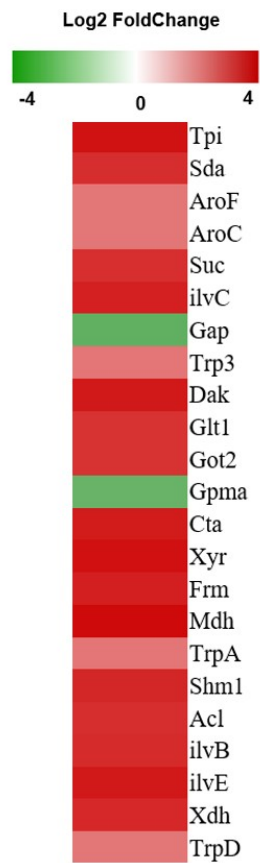
Supplemental Fig.1 Subcellular localization of the eGFP fused with peroxisomal targeting signals SKL.



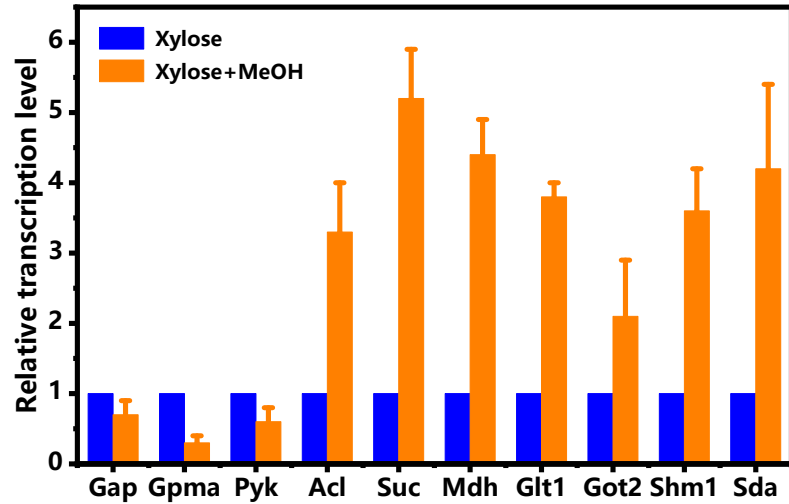
Supplemental Fig. 2 Cell growth of Y1-002 in SD medium supplemented with 20 g/L of xylose (A) and SAE (B).



Supplemental Fig. 3 Classification of differentially expressed genes according to KEGG_B_class annotation (A) and GO_Term (B).



Supplemental Fig. 4 The heatmap of genes involved in methanol metabolism and showed significant expression. Upregulated and Downregulated genes are indicated with red and green.



Supplemental Fig. 5 Relative transcription level of genes involved in glycolysis (*gap*, *gpma* and *pyk*), TCA cycle (*acl*, *suc* and *mdh*) and amino acids synthesis pathways (*glt1*, *got2*, *shm1* and *sda*) of strain Y1-004 grown on SD medium containing 20 g/L of xylose with or without 20 g/L of methanol.