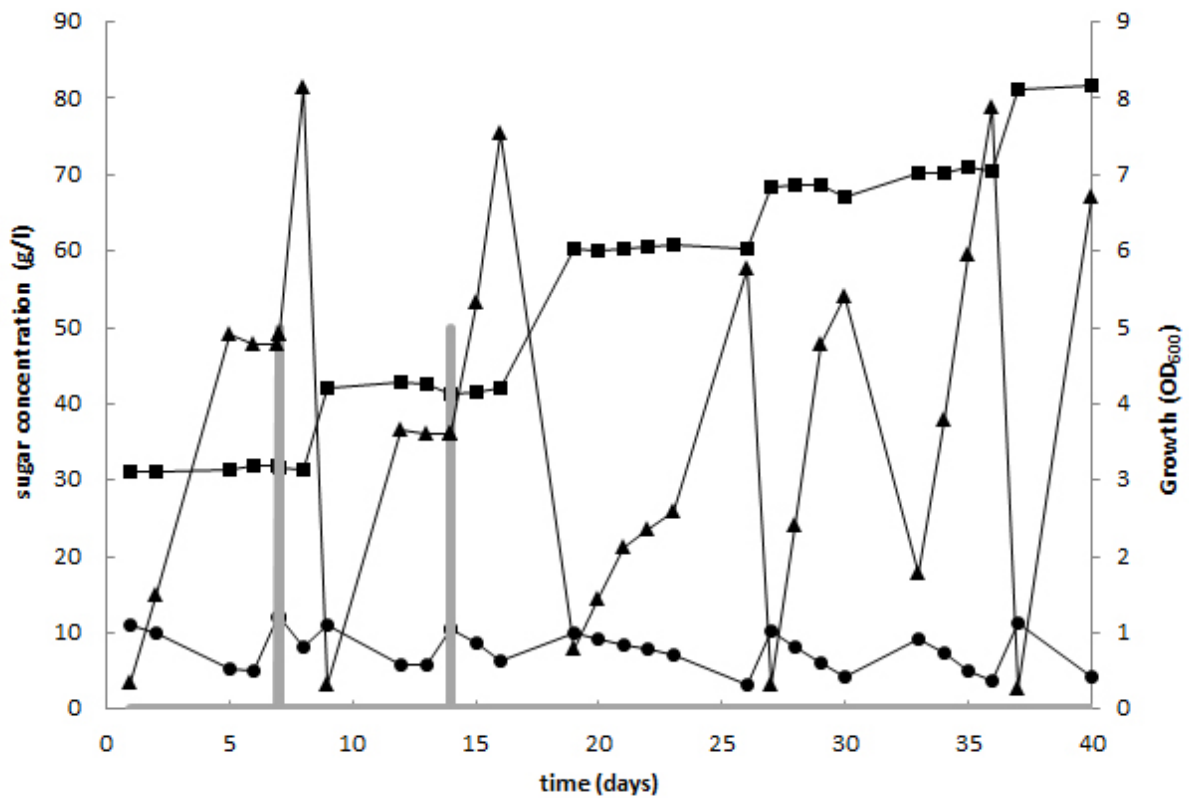
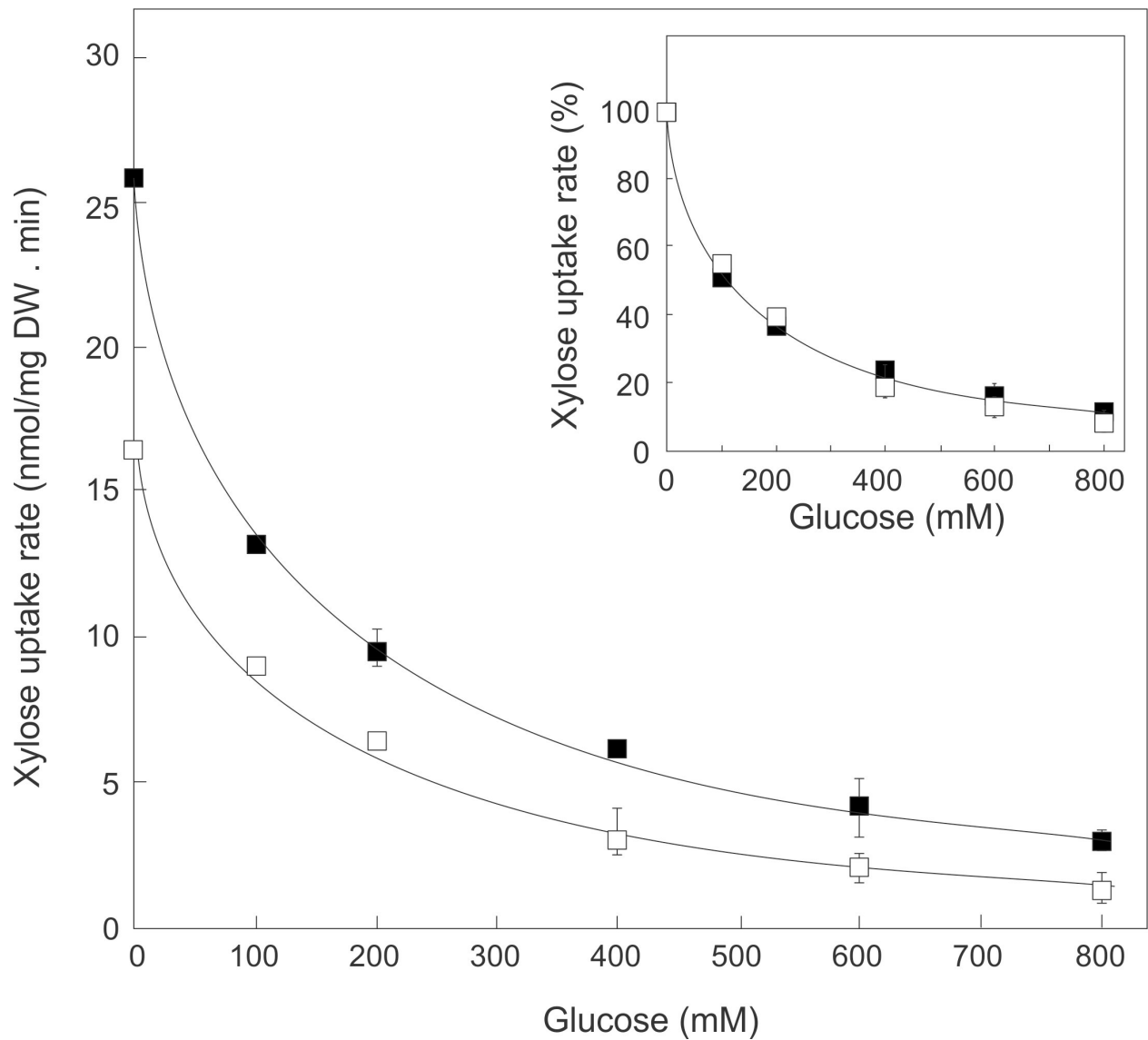


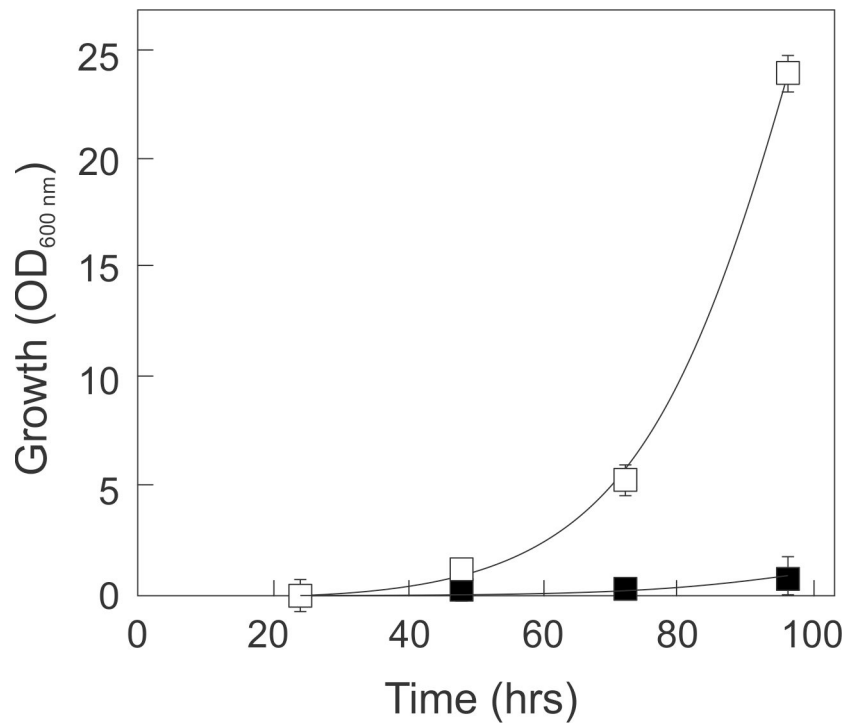
Supplemental data



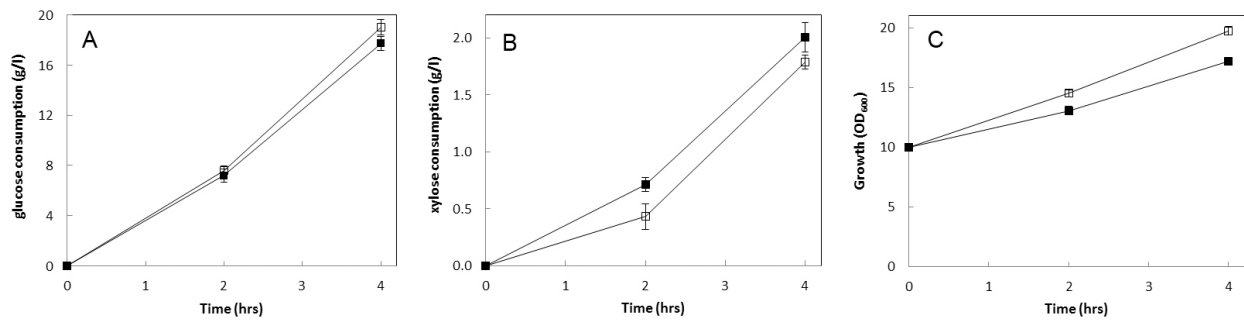
Supplemental Figure 1. Evolutionary engineering process of the DS69473 strain. Depicted are the D-xylose (●) and D-Glucose (■) concentration and the OD₆₀₀ (▲) in time. The grey line indicates the addition of 5 ml of 50% D-xylose added to 500 ml fermentor volume.



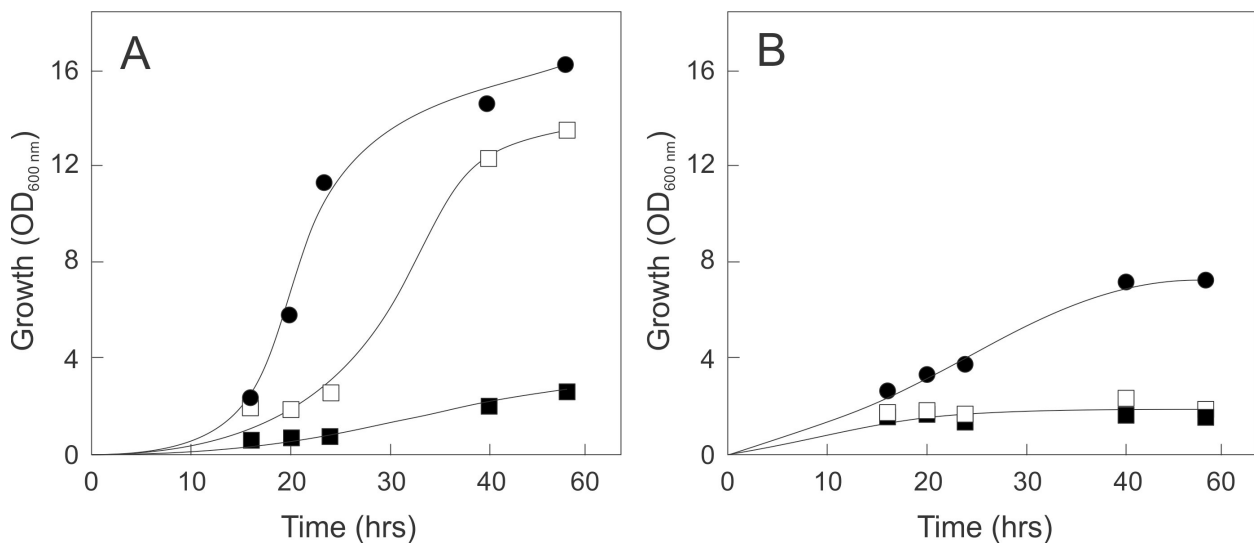
Supplemental Figure 2. Growth (μ , -h) of the DS69473 strains expressing the various Cyc8 Y353 mutants in 2 % D-xylose and 12 % D-glucose after 48 hours in aerobic cultivation. The black bars indicate the Y353C mutant and the wild-type Y353Y, respectively. Error bars were obtained from biological duplicates



Supplemental Figure 3. Normalized fold expression of the *ADHI*, *FIT2*, *HXT10*, *HXT13* and *HXT15/16* genes. Gene expression was analyzed by qPCR using cells grown on minimal medium with 2% D-xylose and 6% D-glucose for the wild-type DS68616 strain (black bars), the DS68616 *CYC8* Y353C mutant strain (white bars) and the DS68616 Δ *CYC8* strain (grey bars). Fold expression was normalized relative to the *ACT1* expression in the wild-type DS68616 strain, which was set to 1.



Supplemental Figure 4. Glucose consumption (panel A), xylose consumption (panel B) and growth (panel C) during mixed sugar fermentation of DS68616-CYC8-Y353C (■) and the wild-type DS68616-CYC8-Y353 (□) on 2% D-glucose and 2% D-xylose.



Supplemental Figure 5. Growth (OD₆₀₀) of the DS68625-derived strains on 2% D-glucose (panel A) and 2% D-xylose + 0.05% D-maltose (panel B). Depicted are strain DS68625 with a plasmid expressing HXT10 (●), HXT15 (□), or the empty vector pRS313-P7T7 (■).

Supplemental Table 1. Genomic sequencing data. Mapping was done against CEN.PK113-7D and the strain DS69473 and DS69473Evo were compared. MNV, multinucleotide polymorphisms; SNP, single nucleotide polymorphisms.

Type	Ref	Allele	Note	Coding region change	Amino acid change	CDS	Annotation
SNP	A	C	None reliable near telomere				
Deletion	T	-	None reliable long stretch of Ts				
Deletion	A	-	Low coverage				
SNP	T	C		1058A>G	Y353C	Cyc8p	General transcriptional co-repressor
Deletion	A	-	None reliable long stretch of As				
Insertion	-	T	None reliable long stretch of Ts	1740_1741insA	L581fs	Pol4p	DNA polymerase IV
Deletion	TA	-	Near assembly gap				
Insertion	-	TT	Also present in parent				
MNV	TT	GC	Also present in parent				
Insertion	-	GC	Near assembly gap				
Insertion	-	T	None reliable long stretch of Ts				
Deletion	T	-	None reliable long stretch of Ts				
Deletion	C	-	None reliable long stretch of Ts				
Insertion	-	A	None reliable long stretch of As				
MNV	AA	GG	Low coverage. caused by too large deletion in this strain	1754_1755del TTinsCC	F585S	Rtg2p	Sensor of mitochondrial dysfunction
Deletion	T	-	Low coverage				
SNV	A	G	Also present in parent				
Deletion	AA	-	Also present in parent				
Deletion	A	-	Also present in parent				
Insertion	-	T	Also present in parent				
Insertion	-	A	None reliable long stretch of As				
Insertion	-	T	Also present in parent				
Insertion	-	G	Also present in parent				
Insertion	-	T	Also present in parent				
Deletion	G	-	Also present in parent				
Insertion	-	T	Also present in parent				
SNP	A	T	None reliable long stretch of Ts				
Deletion	C	-	None reliable long stretch of Ts				
Insertion	-	A	None reliable long stretch of As				
SNP	C	A	Near assembly gap	432G>T		Fsh2p	Putative serine hydrolase
SNP	A	T	Near assembly gap	289+2T>A			
Insertion	-	T	Also present in parent	1_2insA	Met1?	Kre1p	Cell wall glycoprotein
Insertion	-	T	None reliable long stretch of Ts				
Insertion	-	A	None reliable long stretch of As				
Deletion	A	-	Also present in parent				
Insertion	-	T	Also present in parent				
Deletion	A	-	Near assembly gap				
Deletion	A	-	Also present in parent				
MNV	GGG	AAA	Also present in parent	639_641delGG GinsAAA	P213_G214d elinsP_K	Spt14p	UDP-GlcNAc-binding and catalytic subunit
Insertion	-	T	Also present in parent				
Deletion	T	-	Also present in parent				

Supplemental Table 2. Summary of transcriptomics data. Depicted genes were quantified and run in an intensity difference statistical ($p < 0.05$) and subsequently ranked based on fold change (FC) between DS69473 Y353 and DS69473 Y353C.

Probe	Gene	Chr	P-value	Annotation	Y353 avg	Y353C avg	FC
YOL161C	PAU20	XV	0.000	Protein of unknown function (seripauperin multigene family)	0.1	21.7	217.0
YBR301W	PAU24	II	0.000	Cell wall mannoprotein; has similarity to Tir1p	0.7	50.3	75.5
YIR019C	FLO11	IX	0.000	GPI-anchored cell surface glycoprotein (flocculin)	65.3	4695.0	71.9
YCR104W	PAU3	III	0.003	Protein of unknown function (seripauperin multigene family)	0.3	21.7	65.0
YEL069C	HXT13	V	0.000	Hexose transporter	10.7	367.3	34.3
YMR325W	PAU19	XIII	0.000	Protein of unknown function (seripauperin multigene family)	6.0	196.0	32.7
YDR039C	ENA2	IV	0.000	Na(+)-exporting P-type ATPase ENA2	24.3	742.0	30.5
YPL282C	PAU22	XVI	0.000	Protein of unknown function (seripauperin multigene family)	3.0	85.0	28.3
YDR038C	ENA5	IV	0.000	Na(+)-exporting P-type ATPase ENA5	30.7	839.3	27.4
YER011W	TIR1	V	0.000	Cell wall mannoprotein; Srp1p/Tip1p family	8.7	213.0	24.6
YBL108C-A	PAU9	II	0.012	Protein of unknown function (seripauperin multigene family)	1.0	24.3	24.3
YCR010C	ADY2	III	0.000	Acetate transporter required for normal sporulation	53.7	1286.3	24.0
YJR150C	DAN1	X	0.000	Cell wall mannoprotein; has similarity to Tir1p	24.7	584.7	23.7
YOR394W	PAU21	XV	0.000	Protein of unknown function (seripauperin multigene family)	4.0	87.7	21.9
YDR040C	ENA1	IV	0.000	Na(+)/Li(+)-exporting P-type ATPase ENA1 PMR2 HOR6	41.7	857.3	20.6
YIR041W	PAU15	IX	0.006	Protein of unknown function (seripauperin multigene family)	1.7	31.0	18.6
YHR139C	SPS100	VIII	0.000	Protein required for spore wall maturation	12.3	178.3	14.5
YGR294W	PAU12	VII	0.018	Protein of unknown function (seripauperin multigene family)	4.7	55.3	11.9
YNL117W	MLS1	XIV	0.007	Malate synthase. enzyme of the glyoxylate cycle	214.3	2308.7	10.8
YIL162W	SUC2	IX	0.002	Invertase; sucrose hydrolyzing enzyme	1194.0	12521.7	10.5
YDL246C	SOR2	IV	0.013	Protein of unknown function; 99% identical to the Sor1p	128.0	1291.7	10.1
YJR159W	SOR1	X	0.005	Sorbitol dehydrogenase	115.0	1151.0	10.0
YMR175W	SIP18	XIII	0.002	Phospholipid-binding hydrophilin	14.0	136.3	9.7
YDR536W	STL1	IV	0.007	Glycerol proton symporter of the plasma membrane	123.3	1179.3	9.6
YGR088W	CTT1	VII	0.031	Cytosolic catalase T	134.0	1212.0	9.0
YDL245C	HXT15	IV	0.003	Hexose transporter	54.0	485.0	9.0
YOR382W	FIT2	XV	0.034	Mannoprotein that is incorporated into the cell wall	254.3	2210.7	8.7
YNR060W	FRE4	XIV	0.000	Ferric reductase	47.3	401.7	8.5
YOR391C	HSP33	XV	0.000	Possible chaperone and cysteine protease	22.7	191.7	8.5
YJR158W	HXT16	X	0.000	Hexose transporter	38.0	315.7	8.3
YPL272C	PB1	XVI	0.035	Putative protein of unknown function	145.3	1200.3	8.3
YFLO11W	HXT10	VI	0.014	Hexose transporter	15.7	127.0	8.1
YMR322C	SNO4	XIII	0.009	Possible chaperone and cysteine protease	17.0	130.7	7.7
YOR383C	FIT3	XV	0.016	Mannoprotein that is incorporated into the cell wall	760.7	5707.7	7.5
YNR073C		XIV	0.000	Putative mannitol dehydrogenase; paralog of DSF1	37.7	276.0	7.3
YEL039C	CYC7	V	0.000	cytochrome c isoform 2 iso-2-cytochrome c	25.0	182.3	7.3
YLR307C-A		XII	0.014	Putative protein of unknown function	176.7	1261.7	7.1
YEL070W	DSF1	V	0.000	Putative mannitol dehydrogenase	41.7	296.0	7.1
YER053C-A		V	0.037	Protein of unknown function	11.0	74.7	6.8
YMR244W		XIII	0.020	Putative protein of unknown function	141.0	942.7	6.7
YER065C	ICL1	V	0.041	Isocitrate lyase	1288.0	8385.3	6.5
YDR133C		IV	0.009	Dubious open reading frame	650.7	4216.0	6.5
YOL084W	PHM7	XV	0.000	Protein of unknown function	56.3	364.3	6.5
YAR070C		I	0.024	Dubious open reading frame	5.3	34.0	6.4
YGR067C		VII	0.000	Putative protein of unknown function	68.0	433.0	6.4
YIL011W	TIR3	IX	0.006	Cell wall mannoprotein; Srp1p/Tip1p family	112.0	704.3	6.3
YAL062W	GDH3	I	0.021	glutamate dehydrogenase (NADP(+)) GDH3 FUN51	186.0	1166.0	6.3
YPL280W	HSP32	XVI	0.000	Possible chaperone and cysteine protease	24.7	145.3	5.9
YMR095C	SNO1	XIII	0.004	Protein of unconfirmed function	92.7	545.7	5.9
YBR117C	TKL2	II	0.031	Transketolase; paralog of TKL1	78.3	461.0	5.9
YJR094C	IME1	X	0.000	Master regulator of meiosis	15.7	91.7	5.9
YDR070C	FMP16	IV	0.000	Protein of unknown function	41.3	235.3	5.7
YLR154C-H		XII	0.027	Putative protein of unknown function; paralog of YLR157C-C	141.7	801.3	5.7
YPL223C	GRE1	XVI	0.006	Hydrophilin essential in desiccation-rehydration process	127.7	706.3	5.5
YEL049W	PAU2	V	0.003	Member of the seripauperin multigene family	59.0	324.3	5.5
YOL150C		XV	0.001	Dubious open reading frame	47.0	250.0	5.3
YBR072W	HSP26	II	0.047	Small heat shock protein (sHSP) with chaperone activity	479.0	2540.3	5.3
YER096W	SHC1	V	0.000	Sporulation-specific activator of Chs3p (chitin synthase III)	44.0	220.3	5.0

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YGL158W	RCK1	VII	0.023	Protein kinase involved in oxidative stress; paralog of RCK1	10.7	53.3	5.0
YDR534C	FIT1	IV	0.004	Mannoprotein that is incorporated into the cell wall	32.3	161.0	5.0
YNL270C	ALP1	XIV	0.007	Arginine transporter; paralog of CAN1	56.7	281.0	5.0
YIL099W	SGA1	IX	0.005	glucan 1.4-alpha-glucosidase	78.0	380.7	4.9
YIR028W	DAL4	IX	0.006	Allantoin permease	58.3	284.3	4.9
YBR147W	RTC2	II	0.028	Putative vacuolar membrane transporter; paralog of YPQ1	92.7	446.0	4.8
YMR094W	CTF13	XIII	0.013	Subunit of the CBF3 complex	37.3	176.0	4.7
YMR195W	ICY1	XIII	0.015	Protein of unknown function; paralog of ICY2	116.7	549.3	4.7
YGR065C	VHT1	VII	0.034	High-affinity plasma membrane H ⁺ -biotin symporter	288.0	1328.7	4.6
YLL055W	YCT1	XII	0.042	High-affinity cysteine-specific transporter	425.7	1959.0	4.6
YHR137C-A		VIII	0.021	Dubious open reading frame	118.0	536.7	4.5
YHR137W	ARO9	VIII	0.034	aromatic-amino-acid:2-oxoglutarate transaminase	239.7	1058.7	4.4
YIL111W	COX5B	IX	0.023	cytochrome c oxidase subunit Vb	31.3	136.7	4.4
YPR027C		XVI	0.005	Putative protein of unknown function	30.0	130.3	4.3
YOL154W	ZPS1	XV	0.007	Putative GPI-anchored protein	42.0	179.0	4.3
YGL162W	SUT1	VII	0.021	Transcription factor of the Zn(II)2Cys6 family; paralog of SUT2	45.0	186.3	4.1
YDL085W	NDE2	IV	0.007	NADH-ubiquinone reductase (H ⁺)-translocating) NDE2 NDH2	88.7	364.0	4.1
YPL036W	PMA2	XVI	0.009	H ⁺ -exporting P2-type ATPase PMA2	107.3	436.7	4.1
YIL020C	HIS6	IX	0.034	1-(5-phosphoribosyl)-5- ((5-phosphoribosylamino)methylidene	40.0	162.0	4.1
YOR393W	ERR1	XV	0.024	phosphopyruvate hydratase ERR1	25.3	102.0	4.0
YJL089W	SIP4	X	0.007	C6 zinc cluster transcriptional activator	45.3	180.0	4.0
YLR159C-A		XII	0.006	Putative protein of unknown function	15.7	62.0	4.0
YDR354C-A		IV	0.020	Dubious open reading frame	35.7	138.0	3.9
YIL166C		IX	0.015	Putative protein with similarity to allantoate permease	47.3	180.7	3.8
YMR323W	ERR3	XIII	0.007	phosphopyruvate hydratase ERR3	39.7	148.7	3.7
YOR237W	HES1	XV	0.028	oxysterol-binding protein related protein HES1 OSH5	28.0	103.0	3.7
YOR100C	CRC1	XV	0.004	Mitochondrial inner membrane carnitine transporter	79.7	291.7	3.7
YPL281C	ERR2	XVI	0.024	phosphopyruvate hydratase ERR2	26.3	95.0	3.6
YGR144W	THI4	VII	0.005	thiamine thiazole synthase MOL1 ESP35	37.0	133.0	3.6
YGR052W	FMP48	VII	0.008	Putative protein of unknown function	29.7	103.7	3.5
YBL075C	SSA3	II	0.041	Hsp70 family ATPase SSA3 YG106	98.7	339.7	3.4
YDL023C		IV	0.040	Dubious open reading frame	50.7	168.0	3.3
YJR025C	BNA1	X	0.037	3-hydroxyanthranilate 3.4-dioxygenase HAD1	178.3	565.7	3.2
YMR194C-B	CMC4	XIII	0.047	Protein localizes to the mitochondrial intermembrane space	43.7	125.0	2.9
YIR032C	DAL3	IX	0.042	ureidoglycolate hydrolase	44.7	126.7	2.8
YBR299W	MAL32	II	0.031	alpha-glucosidase MAL32 MALS MAL35	25252.7	3741.0	0.1
YBR093C	PHO5	II	0.000	Repressible acid phosphatase	675.0	74.3	0.1

Supplemental Table 3. Strains and plasmids used in this study

Strain/ plasmid	Relevant genotype and/or characteristics	Source/reference
Strains		
<i>S. cerevisiae</i>		
DS68616	<i>Mat a. ura3-52. leu2-112. gre3::loxP. loxP-Ptpi:TAL1. loxP-Ptpi::RKII. loxP-Ptpi-TKL1. loxP-Ptpi-RPE1. delta::Padh1XKSITcyc1-LEU2. delta::URA3-Ptpi-xylA-Tcyc1. His3::LoxP</i>	DSM. The Netherlands
DS68625	DS68616. <i>his3::loxP. hxt2::loxP-kanMX-loxP. hxt367::loxP-hphMX-loxP. hxt145::loxP-natMX-loxP. gal2::loxP-zeoMX-loxP</i>	[1,2]
DS69473	DS68616 <i>his3::loxP glk1::lox72 hxk1::loxP-hphMX-loxP hxk2::lox72 gal1::loxP</i>	This paper
DS69473Evo	DS69473-derivative after evolutionary engineering by chemostat cultivation on xylose in presence of glucose	This paper
Plasmids		
pRS313	<i>E. coli</i> /yeast shuttle vector; <i>CEN6. ARSH4. HIS3. Amp^r</i>	[3] This paper
pRS313-P7T7_Hxt10	pRS313 containing the promotor and terminator of HXT7 [1] and expressing HXT10	This paper
pRS313-P7T7_Hxt15	pRS313 containing the promotor and terminator of HXT7 [1] and expressing HXT15	

Supplemental Table 4. Oligonucleotides used in cloning and sequencing.

Name	Sequence (5' → 3')
Hexokinase deletion strain	
GLK1-psuc227f	TATCACGTGCAGCCCAGGATAATTTTCAGGACACGTGTTTCGAAAGGTTTGT CGCTCCGATCGACCTCGAGTACCGTTTCG
GLK1-psuc225r	ATTTAGTGAGCTGTTTCTTGTCAAAAACAACCAACGGAAGAGGGGCGAGGCTGT TTCTCCGCGGATCCTACCGTTCGTATAG
HXK2-psuc227f	CCACGAAATTACCTCCTGCTGAGGCGAGCTTGCAAATATCGTGTCCAATTCC GTGATGTCTCGACCTCGAGTACCGTTTCG
HXK2-psuc225r	TACAAAAGAAAGTACGCAAGCTATCTAGAGGAAGTGTAGAGAGGGTTAAAA TTGGCGTGCCGATCCTACCGTTCGTATAG
HXK1_loxP_f	TCGGTTTCACTTCTTGGGAATATTCTACCGTTCCTTCATCTTGTATTCCGGAT CCACTAGCATAACTTCG
HXK1_loxP_r	GACAATGCAGCAATAACAGCAGCACCTGCACCTGAACCATCCTCAGCTTTGG GCCGCCAGTGTGATGG
GAL1_loxP_f	TGTGCCTCGCGCCGACTGCTCCGAACAATAAAGATTCTACAATACTAGCGG ATCCACTAGCATAACTTCG
GAL1_loxP_r	AGGTATCCAAAACGCAGCGGTTGAAAGCATATCAAGAATTTTGTCCCTGTTT GGGCCGCCAGTGTGATGG
CYC8 Y353C mutation	
F kanMX 5'tail	CCCTTTTCTTCTTTTGTGCACCACTCAGGGCACGAGAAAAGCTT ATTTTTTTGTAACGGCCGCCA
R KanMX + Cyc8	CCACTAAGAACGGAGTGTGCACGCTAGATATCGTCGACACTGGATGGC
F KanMX + Cyc8	GCCATCCAGTGTGACGATATCTAGCGTGCACACTCCGTTCTTAGTGG
R Cyc8	GTACCTAGATCGTACCAAACCTTCAC
F Cyc8	GGAAGCCTACGAGCATGTCTTGG
CYC8 Y353X Crispr/Cas9	
F KanMX MunI	GATCCAATTGAGCTTGCCTCGTCCCCGCCG
R KanMX Adel	CCTTCACGTAGTGTGACACTGGATGGCGGCGTTAG
targetRNA F Cyc8	TGCGCATGTTTCGGCGTTTCGAACTTCTCCGCAGTGAAAGATAAATGATCGG <u>CTTTGTGTACGCGTCTAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG</u> CTAGTCCGTTATCAAC
targetRNA R Cyc8	GTTGATAACGGACTAGCCTTATTTAACTTGCTATTTCTAGCTCTAAAAC <u>TAG</u> <u>ACGCGTACACAAGAGCCGATCATTTATCTTTCACTGCGGAGAAGTTTCGAAC</u> GCCGAAACATGCGCA
F repair Cyc8 WNN	GTTTTATATTACCAAATTTCTCAATACAGAGACGCTTTAGACGCGWNNACAA GAGCCATAAGATTAATCCTTATATTAGTGAAGTTTGG
R repair Cyc8 WNN	CCAACTTCACTAATATAAGGATTTAATCTTATGGCTCTTGTNNWCGGTCT AAAGCGTCTCTGTATTGAGAAATTTGGTAATATAAAAC
F repair Cyc8 SNN	GTTTTATATTACCAAATTTCTCAATACAGAGACGCTTTAGACGCGSNNACAA GAGCCATAAGATTAATCCTTATATTAGTGAAGTTTGG
R repair Cyc8 SNN	CCAACTTCACTAATATAAGGATTTAATCTTATGGCTCTTGTNNSCGCGTCTA AAGCGTCTCTGTATTGAGAAATTTGGTAATATAAAAC
CYC8 deletion	
F 5'FR cyc8 kanMX	TACAACTACAACAGCAACAACAACAACAAAACACGACTGGAAAAAAAAAAAA TTAGGAAAAAGCTTGCCCTCGTCCCCGCCGGG
R 3'FR cyc8 kanMX	GCTACACAACATTTCTCGTTGATTATAAATTAGTAGATTAATTTTTTGAATGC AAACTTTCTAGATATCGTCGACACTGGATGGCGG

***HXT* Overexpression**

R Hxt10 XbaI	<i>ATGCTCTAGA</i> ATGGTTAGTTCAAGTGTTCATTTGGG
R Hxt10 Cfr9I	GACTCCCGGGTATTTACTATCAACAATAACTAATGGTGTACTGCTTGTGGT TGTGGTGTTCCTAGAACTGG
F Hxt15 XbaI	ATCGATCTAGAATGGCAAGCGAACAGTCCTCACC
R Hxt15 Cfr9I	TAGACCCCGGGTCAATTAAAACCTTTGGGAACCTCAA

Italic: restriction enzyme site

Underlined: sequence of the targets in *CYC8*

Bold: Codon 353 in *CYC8*

Supplemental Table 5. Oligonucleotides used in qPCR.

Name	Sequence (5' → 3')
ActinF	GGATTCTGAGGTTGCTGCTTTGG
ActinR	GAGCTTCATCACCAACGTAGGAG
HXT1F	TGTTCTCTGTACACCGTTGACCG
HXT1R	AGATCATAACAGTTACCAGCACCC
HXT2F	CTTCGCATCCACTTTCGTG
HXT2R	AATCATGACGTTACCGGCAGCC
HXT3F	GAAGCTAGAGCTGCTGGTTCAGC
HXT6R	ACAACGACATAAGGAATTGGAGCC
HXT4F	ATGGAGAGTTCCATTAGGTCTAGG
HXT4R	ATAACAGCTGGATCGTCTGCGC
HXT5F	TTGCTATGTCGTCTATGCCTCTG
HXT5R	AGATAAGGACATAGGCAACGGG
HXT7F	GGGTGCTGCATCCATGACTGC
HXT7R	ACAACGACATAAGGAATTGGAGCC
HXT8F	GTACTACTATCTTCAAATCTGTCTGG
HXT8R	CTTGTGACGCCAACGGAGGCC
HXT9F	CCATTGAGAGGTTTGGACGCCG
HXT9R	ACACAATCATAACAGTTACCGGCCG
HXT10F	GGAATGCAAGACTCTTTCGAGAC
HXT10R	CTAGTGACGCCAACGGTGGCG
HXT11F	GCCACTCAATGGAGAGTCGGC
HXT11R	CAACTAGCAAGGCTGGATCGTC
HXT12F	CACCATCTTCAAATCTGTCTGGTC
HXT12R	CAATCATAACAGTTACCGGCACCC
HXT13F	CCCTCATGGCCAGGACGGTC
HXT13R	TTGCCATAACCAGTTGCATGCAG
HXT14F	GCCTTAGTAGTGTACTGCATCGGT
HXT14R	TGATACGTAGATACCATGGAGCC
HXT15F	GAGGCCTGTGTCTCCATCGCC
HXT15R	CACAAGAATACCTGTGATCAAACG
HXT16F	CAAGGAAGTATAGTAATACTGCGC
HXT16R	TTGGCGATGGAGACACAGGCC
GAL2F	TCAATGGAGAGTTCCATTAGGGC
GAL2R	CTGGACGGCAGGATCCTCTGG
ADH1 F	CAGATCCATCGGTGGTGAAGTC
ADH1 R	CTCTGGTGTGACGCTCTGTTACC
FIT2 F	CACTAAGGTCGTTACCGACAC
FIT2 R	GCTTGAGTGACGGTCTTGGTG
Cas9F	TCTGCTGGCCCAGATCGGC
Cas9R	GCAGTTGCTGTCTGACAAGGG

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3. Sikorski RS, Hieter P: **A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*.** *Genetics*. 1989, **122**:19-27.