Supplementary data

Table S1. List of nonsynonymous SNPs identified in the fifth-passage strain

Chromosome	Gene	Nucleotide change	Amino acid change	Chromosome	Gene	Nucleotide change	Amino acid change
II	*POP4* *(YBR257W)*	A^{41}→C G^{314}→A	Lys^{14}→Thr Arg^{105}→Lys	XII	*IRC20* *(YLR247C)*	G^{148}→A	Gly^{50}→Ser
V	*DDI1* *(YER143W)*	G^{182}→A	Arg^{61}→Lys		*CTS1* *(YLR286C)*	C^{1300}→A	Leu^{434}→Ile
VIII	*NEL1* *(YHR035W)*	G^{1234}→A	Gly^{412}→Arg		*YLR287C*	G^{1001}→A	Cys^{334}→Tyr
	BRL1 *(YHR036W)*	G^{788}→A	Arg^{263}→Lys		*MEC3* *(YLR288C)*	G^{526}→A	Asp^{176}→Asn
	SMF2 *(YHR050W)*	T^{388}→C	Leu^{130}→Phe		*YLR297W*	G^{185}→C	Arg^{62}→Pro
	RRP3 *(YHR065C)*	G^{607}→A	Val^{203}→Ile		*IMH1* *(YLR309C)*	C^{2486}→G	Ala^{829}→Gly
	SPL2 *(YHR136C)*	C^{4}→G	Arg^{2}→Gly	XIII	*YML131W*	G^{825}→T	Glu^{275}→Asp
IX	*YIL092W*	G^{264}→A G^{1086}→A	Met^{88}→Ile Met^{362}→Ile	XIV	*SWT21* *(YNL187W)*	G^{580}→A	Val^{194}→Met
	FYV10 *(YIL097W)*	G^{465}→C	Lys^{155}→Asn		*CNM67* *(YNL225C)*	G^{338}→A	Gly^{113}→Asp
	CSM2 *(YIL132C)*	G^{530}→A	Arg^{177}→Lys		*JJJ1* *(YNL227C)*	C^{1621}→T	Pro^{541}→Ser
	SLN1	GG^{2217}→AA	Asp^{740}→Asn	XV	*TAT2*	C^{148}→A	His^{50}→Asn

	(YIL147C)	G^{3416}→A	Ser^{1139}→Asn	*(YOL020W)*	A^{1526}→G	Asp^{509}→Gly
	MLP2	A^{74}→G	Lys^{25}→Arg	*MDM38*	G^{1360}→A	Glu^{454}→Lys
	(YIL149C)	G^{2426}→A	Gly^{809}→Asp	*(YOL027C)*		
X	*MDE1*	G^{301}→A	Asp^{101}→Asn	*YOL036W*	C^{855}→G	Asn^{285}→Lys
	(YJR024C)	G^{355}→C	Val^{119}→Leu			
	DAN1	C^{210}→G	Asn^{70}→Lys	*INP54*	A^{345}→T	Lys^{115}→Asn
	(YJR150C)			*(YOL065C)*		
XII	*GRC3*	G^{511}→A	Asp^{171}→Asn	*ATG34*	G^{1153}→A	Val^{385}→Met
	(YLL035W)			*(YOL083W)*		
	YLR230W	C^{226}→G	Pro^{76}→Ala	*PHM7*	G^{1126}→A	Gly^{376}→Ser
				(YOL084W)		

Table S2. *Saccharomyces cerevisiae* strains and plasmids used in this study

Name	Description	Reference
Strains		
D452-2	*MATα, leu2, his3, ura3 and can1*	[1]
POP4_1*	D452-2 with the *POP4*^{K14T} mutation	In this study
POP4_2*	D452-2 with the *POP4*^{R105K} mutation	In this study
DDI1*	D452-2 with the *DDI1*^{R61K} mutation	In this study
RRP3*	D452-2 with the *RRP3*^{V203I} mutation	In this study
MDE1*	D452-2 with the *MDE1*$^{D101N, V119L}$ mutation	In this study
GRC3*	D452-2 with the *GRC3*^{D171N} mutation	In this study
SWT21*	D452-2 with the *SWT21*^{V194M} mutation	In this study
JJJ1*	D452-2 with the *JJJ1*^{P541S} mutation	In this study
TAT2_1*	D452-2 with the *TAT2*^{H50N} mutation	In this study
TAT2_2*	D452-2 with the *TAT2*^{D509G} mutation	In this study
ATG34*	D452-2 with the *ATG34*^{V385M} mutation	In this study
Plasmids		
pCas9_AUR	Aur^{R}, p414-*TEF1p-Cas9-CYC1t*, modified Cas9 expression plasmid, Amp^{R}	[2]
pgRNA-*TRP1*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gTRP1-SUP4t*, Amp^{R}	[3]
pgRNA-*POP4_1*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gPOP4_1-SUP4t*, Amp^{R}	In this study
pgRNA-*POP4_2*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gPOP4_2-SUP4t*, Amp^{R}	In this study
pgRNA-*DDI1*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gDDI1-SUP4t*, Amp^{R}	In this study
pgRNA-*RRP3*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gRRP3-SUP4t*, Amp^{R}	In this study
pgRNA-*MDE1*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gMDE1-SUP4t*, Amp^{R}	In this study
pgRNA-*GRC3*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gGRC3-SUP4t*, Amp^{R}	In this study
pgRNA-*SWT21*-HYB	Hyg^{R}, 2 μ origin, *SNR52p-gSWT21-SUP4t*, Amp^{R}	In this study

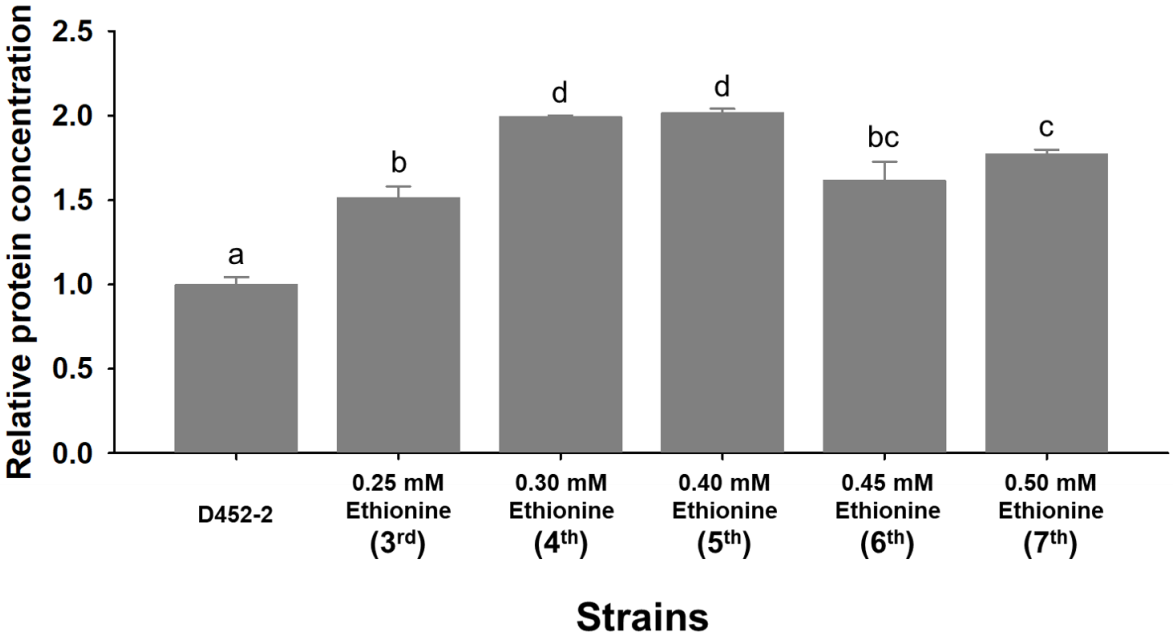
pgRNA-*JJJ1*-HYB	Hyg^R, 2 μ origin, *SNR52p-gJJJ1-SUP4t*, Amp^R	In this study
pgRNA-*TAT2_1*-HYB	Hyg^R, 2 μ origin, *SNR52p-gTAT2_1-SUP4t*, Amp^R	In this study
pgRNA-*TAT2_2*-HYB	Hyg^R, 2 μ origin, *SNR52p-gTAT2_2-SUP4t*, Amp^R	In this study
pgRNA-*ATG34*-HYB	Hyg^R, 2 μ origin, *SNR52p-gATG34-SUP4t*, AmpR	In this study

Table S3. List of primers used in this study

Name	Description	Amplified gene	Description
TH69	TCTTTTCAAGAAATGCCTGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*POP4_1*-HYB
TH70	AACCCAGGCATTTCTTGAAAAGAGATCATTTATCTTTCACTGCGGA		
TH71	CGAAAATAAAATTACTGATAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*POP4_2*-HYB
TH72	AACTATCAGTAATTTTATTTTCGGATCATTTATCTTTCACTGCGGA		
TH152	CCTAATCAATAATAAGTCATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*DDI1*-HYB
TH153	AACATGACTTATTATTGATTAGGGATCATTTATCTTTCACTGCGGA		
TH75	AAATGGTCCATTAATCTACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*RRP3*-HYB
TH76	AACGGTAGATTAATGGACCATTTGATCATTTATCTTTCACTGCGGA		
TH91	AGGAGCATATCACAGCATTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*MDE1*-HYB
TH92	AACAAATGCTGTGATATGCTCCTGATCATTTATCTTTCACTGCGGA		
TH171	TAGAAGCAGGTCATTTATATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*GRC3*-HYB
TH172	AACATATAAATGACCTGCTTCTAGATCATTTATCTTTCACTGCGGA		
TH146	AGTGCACACCTCGATCGCGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*JJJ1*-HYB
TH147	AACCCGCGATCGAGGTGTGCACTGATCATTTATCTTTCACTGCGGA		
TH83	TTATGCGAATGAAGTGTTTCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*SWT21*-HYB
TH84	AACGAAACACTTCATTCGCATAAGATCATTTATCTTTCACTGCGGA		
TH85	TCATCTGTCTCGCCTAGAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*ATG34*-HYB
TH86	AACTTTCTAGGCGAGACAGATGAGATCATTTATCTTTCACTGCGGA		
TH87	ATGACCACCAACACGGCAAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*TAT2_1*-HYB
TH88	AACCTTGCCGTGTTGGTGGTCATGATCATTTATCTTTCACTGCGGA		
TH89	CCGCTGGTCCAGCCACCAATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG	pgRNA-*TRP1*-HYB	To construct pgRNA-*TAT2_2*-HYB
TH90	AACATTGGTGGCTGGACCAGCGGGATCATTTATCTTTCACTGCGGA		
TH108	GGATAGAACTCAAACGTTTATTAAAGAATGTCTTTTCACGAAATGCCTGGA	*POP4_1* repair DNA	To introduce

TH109	AGACCCTGA TCTTGAAATCTATTTTCATTAAAGGGCTTTTCAGGGTCTTCCAGGCATTTCG TGAAAAGA		*POP4_1* SNP
TH110 TH111	ATTGTAAAAAAAATACAAAAAAATGTCTGAAATTGGCATACGAAAATAAAA TTACTGATA TCTCCTCTATGTAGTGTAAAAGGTCTTCTTTATCAGTAATTTTATTTTCGTATG CCAATT	*POP4_2* repair DNA	To introduce *POP4_2* SNP
TH154 TH155	AACAGAACTCAGTCTTTAAAAGAATTAGGGCTCAAAACGGATGACTTATTA TTGATTAGG ATCTGTTTGAATGGAATTGGAAATCTTACCCCTAATCAATAATAAGTCATCCG TTTTGAG	*DDI1* repair DNA	To introduce *DDI1* SNP
TH117 TH118	AGACCTGATGAGAAAGCCACATATTATCATTGCTACAACTGGTAGATTAATG GACCATTT CAGTGAAAACCCTTTGGTATTCTCTAAATGGTCCATTAATCTACCAGTTGTA GCAATGAT	*RRP3* repair DNA	To introduce *RRP3* SNP
TH129 TH130	CCATTGTTCTTAGCGTGCTACCAAAAGAAAAATGCAGGTGCTATAATACATA CACACTCCTAAAATGCTGTGATATGCTCCTTGC TGTTAGCAATCCTGAACTCATCGCCAAATAGCAAGGAGCATATCACAGCATT TTAGGAGTGTGTATGTATTATAGCACCTGCATT	*MDE1* repair DNA	To introduce *MDE1* SNP
TH173 TH174	TTTCCATGTGTTCTTCGCATTTTTAATTCTAATCATACGGGCTTGTTAGAAGC AGGTCATTTATATCGAG GTTCTTTAGGTTTCCATAGATAATTAACATCTCGATATAAATGACCTGCTTCT AACAAGCCCGTATGATT	*GRC3* repair DNA	To introduce *GRC3* SNP
TH123 TH124	AAGCTCTCTCAAGAGGATCTCTATTGTGTGGCAGTTATGCGAATGAAATGTT TCAAGTAG TCAGTCTCTCTAGTCGTTGATGACGGCAGTCTACTTGAAACATTTCATTCGC ATAACTGC	*SWT21* repair DNA	To introduce *SWT21* SNP
TH148 TH149	AGCACTCCGTCCTTATCGACTCTATCGTCCTCTATGTCTCCAACATCCGCGAT CGAGGTGTGCACT ATTTCGACTATCAAATGATTCTCCGCATGTAGTGCACACCTCGATCGCGGAT GTTGGAGACATAGA	*JJJ1* repair DNA	To introduce *JJJ1* SNP
TH119 TH120	TCCTCATCACACAATTCAAACTCATCGCATCACGATGATGACAACCAACAC GGCAAGAGA GAAAGAATCAACACATCGCTGGAAAATATTTCTCTTGCCGTGTTGGTTGTC ATCATCGTG	*TAT2_1* repair DNA	To introduce *TAT2_1* SNP
TH127 TH128	ATCTTGATAGCTCAATTCTATTGTTCACTATGGACCATTGGTGGCTGGACCA GCGGCAAA ATAATTTTGAAAAAATATTTTAGCCCTTTCTTTGCCGCTGGTCCAGCCACCA	*TAT2_2* repair DNA	To introduce *TAT2_2* SNP

	ATGGTCCA		
TH125	GCGCTTTCTTCCCCTGATGAATCAAGCATCATGAGCACTACCTTTCTAGGCG AGACAGATGA	*ATG34* repair DNA	To introduce *ATG34* SNP
TH126	CTACTTAATGTAGAGCCAGAATTATAAACTTCATCTGTCTCGCCTAGAAAGG TAGTGCTCAT		



Supplementary Figure 1. Comparison of relative protein concentration of parental and evolved strains. Relative protein content was determined using the Bradford assay. Results are the mean of three experiments, and error bars indicate the standard deviation. Different letters represent significantly different means (Tukey's honestly significant difference tests, $p < 0.05$).

Reference

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