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## Supplemental Information

# Critical Roles of the Pentose Phosphate Pathway and *GLN3* in Isobutanol-Specific Tolerance in Yeast

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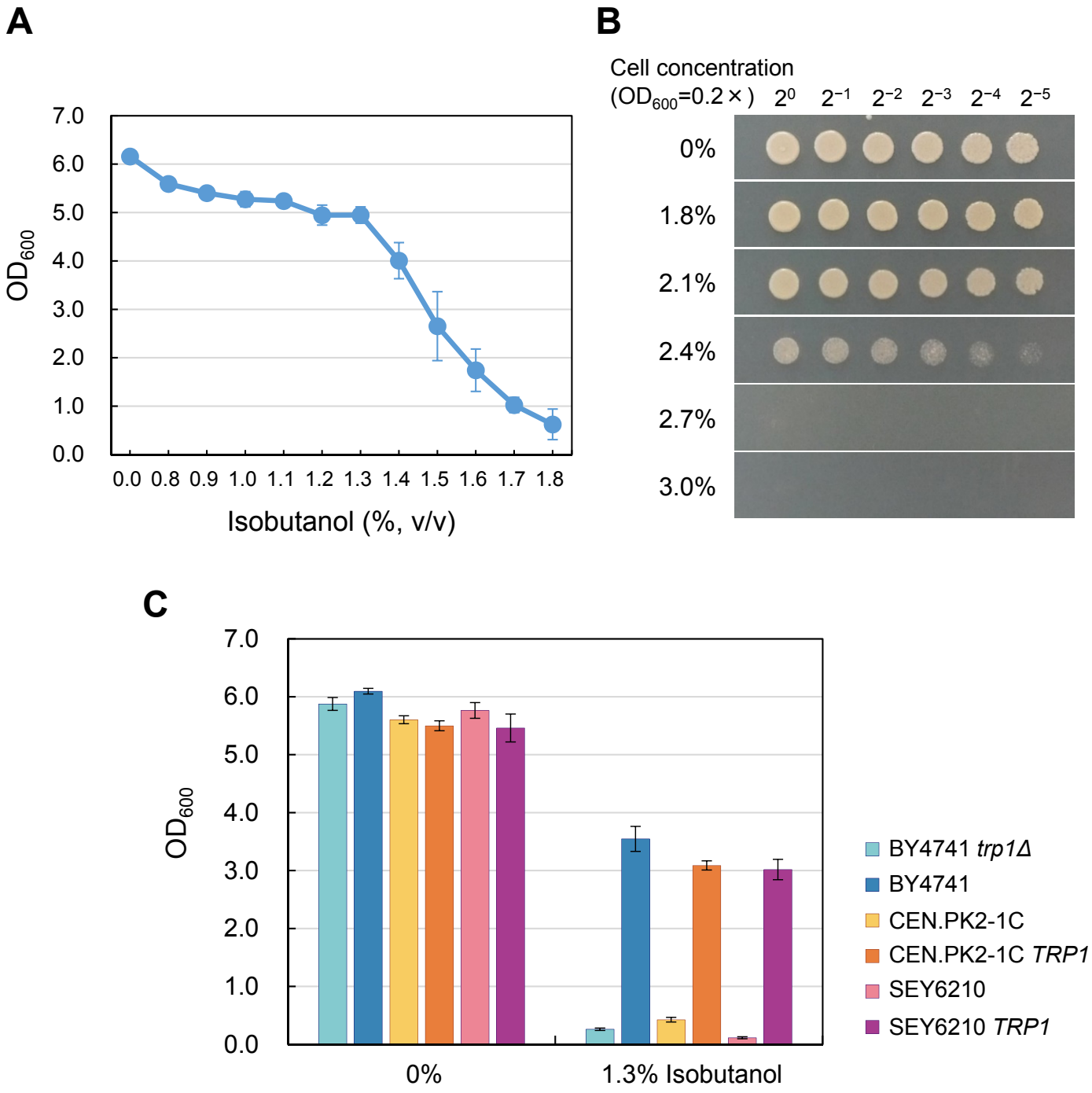


**Figure S1. Cell growth of the BY4741 wild-type strain in synthetic complete (SC) liquid medium containing isobutanol and effect of *trp1Δ* allele in wild-type laboratory strains (related to Figure 1).** Cell growth of the BY4741 wild-type strain was monitored in SC liquid medium (A) and solid medium (B) after 24 h of cultivation at 30°C. (C) Cell growth of the wild-type strains (BY4741, CEN.PK2-1C, and SEY6210) with or without *TRP1* was measured in liquid SC medium containing 1.3% (v/v) isobutanol. Error bars represent the SEM of three independent experiments.

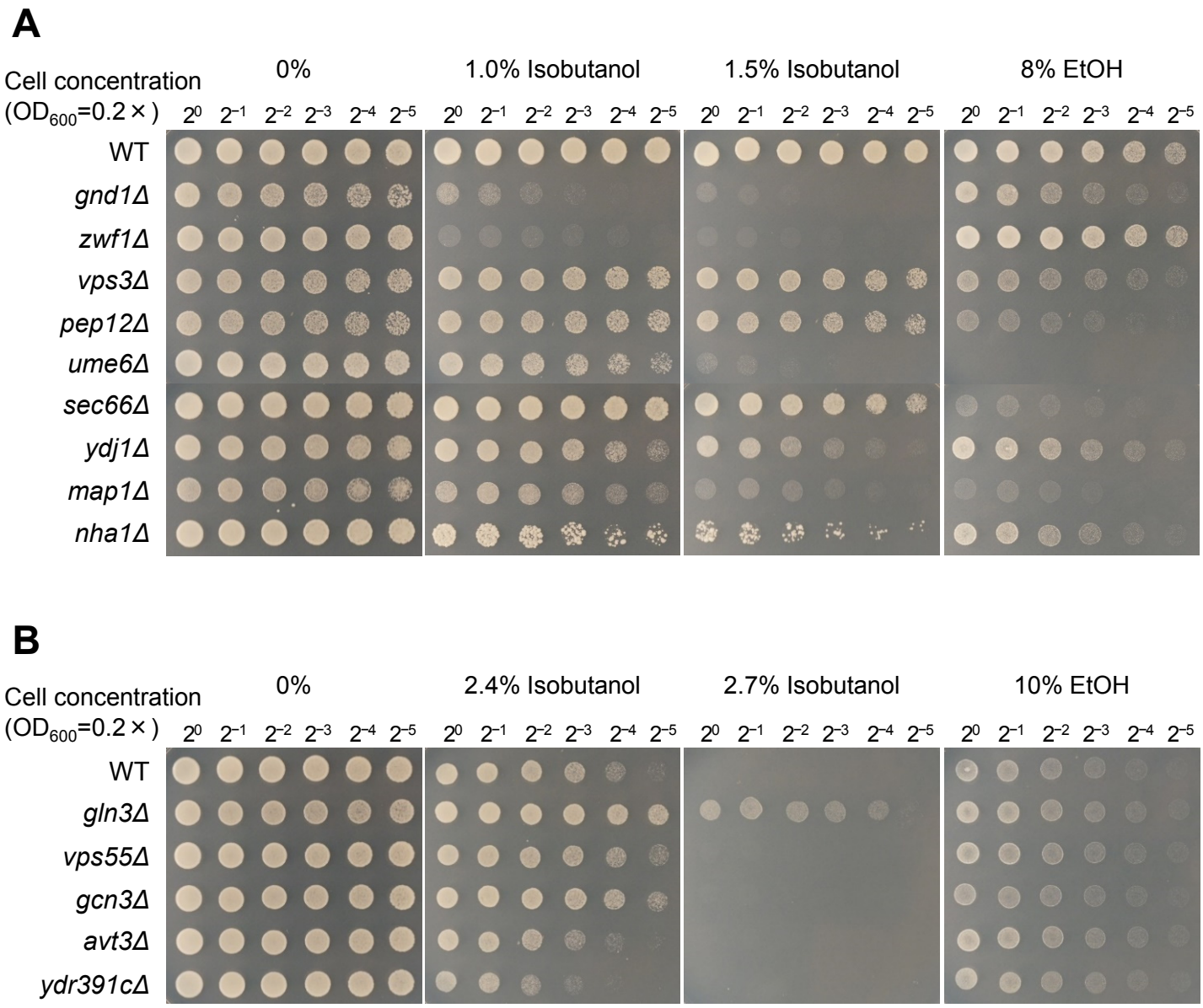


**Figure S2. Cell growth of most hypersensitive and hypertolerant strains to isobutanol in solid medium containing isobutanol or ethanol (related to Figures 2 and 4).** Cell suspensions (10 µL) of isobutanol-hypersensitive strains in Figure 2A (A) and isobutanol-hypertolerant strains (B) were spotted on SC agar plates. Each subsequent spot represents a 2-fold dilution. Plates were incubated at 30°C for 48 h.

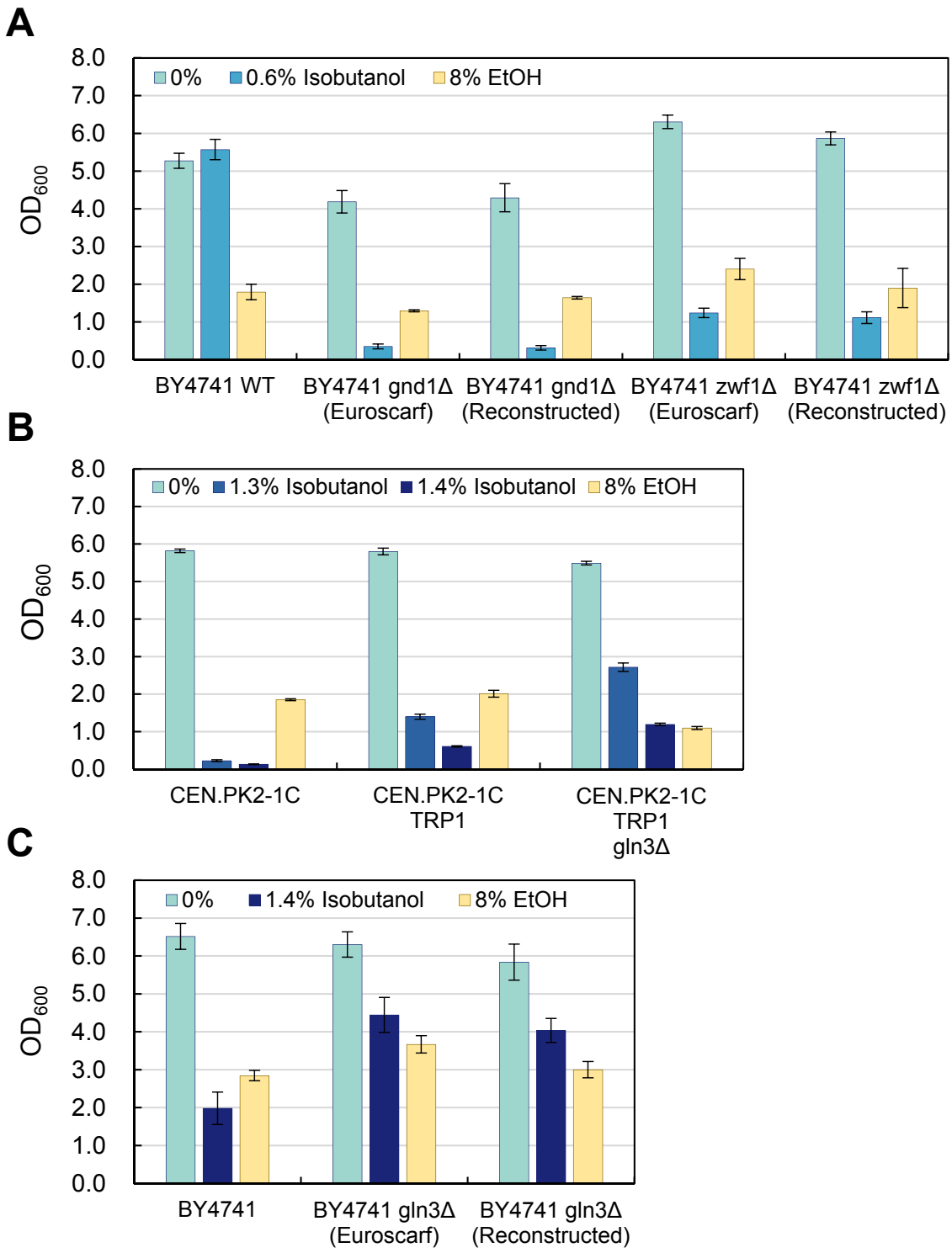


**Figure S3. Isobutanol and ethanol sensitivity of reconstructed deletion strains in BY4741 and CEN.PK2-1C background (related to Figures 2 and 4).** (A) Cell growth of the reconstructed BY4741 *gnd1Δ* and BY4741 *zwf1Δ* strains was measured in liquid SC medium containing 0.6% (v/v) isobutanol or 8% Ethanol. (B) Cell growth of the *TRP1*-restored CEN.PK2-1C strain and its derivative *gln3Δ* strain was measured in liquid SC medium containing 1.3% isobutanol, 1.4% isobutanol, or 8% Ethanol. (C) Cell growth of the reconstructed BY4741 *gln3Δ* strain was measured in liquid SC medium containing 1.4% isobutanol or 8% Ethanol. Error bars represent the SEM of three independent experiments.

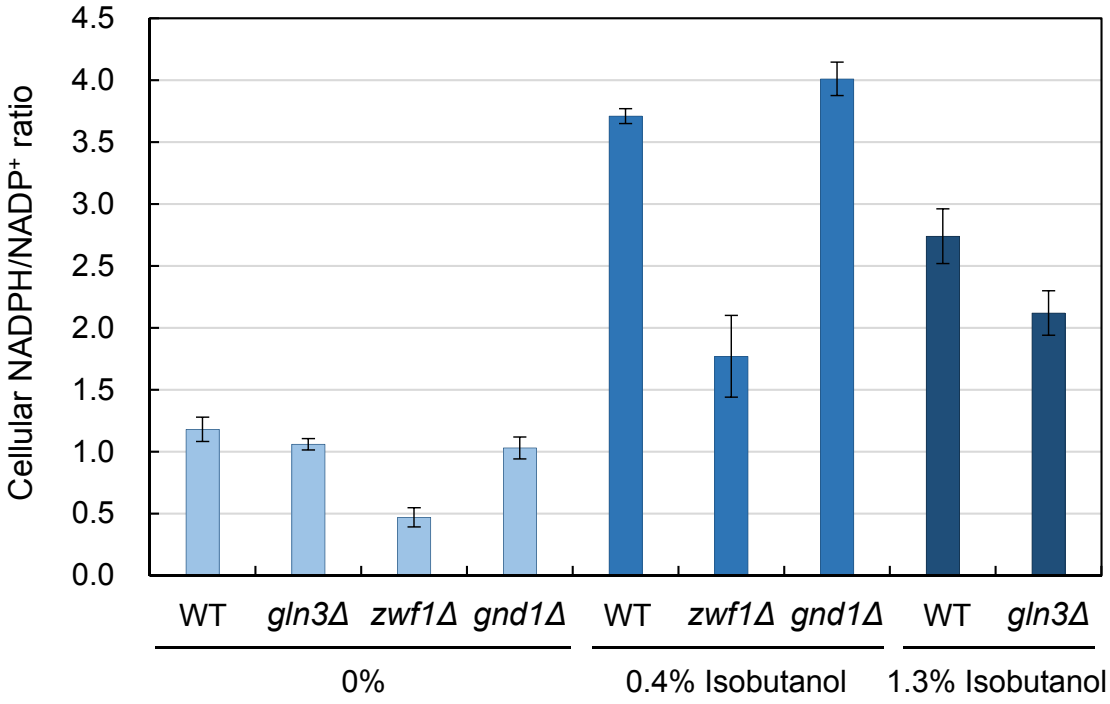


**Figure S4. Cellular NADPH/NADP$^+$ ratios of yeast deletion strains (related to Figures 2 and 3).** Wild type, *gln3Δ*, *zwf1Δ*, and *gnd1Δ* strains were cultivated in liquid SC medium without isobutanol at 30$^o$C for 16 h and SC medium containing 0.4% (v/v) or 1.3% isobutanol at 30$^o$C for 20 h. Error bars represent the SEM of four (for experiments with 0.4% isobutanol), six (for WT and *zwf1Δ* without isobutanol and experiments with 1.3% isobutanol), and eight (for *gln3Δ* and *gnd1Δ* without isobutanol) independent experiments.

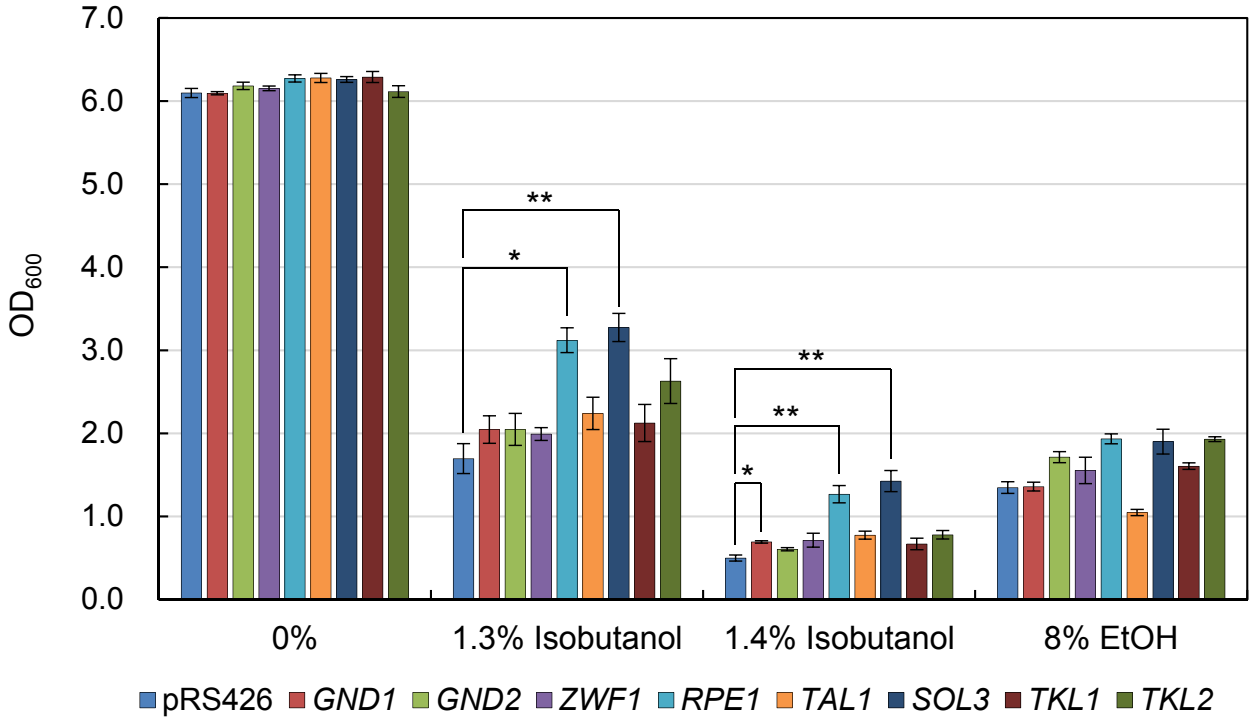


**Figure S5. Isobutanol tolerance of BY4741 strains overexpressing 2μ plasmids with single PPP genes (related to Figures 2 and 3).** Single PPP genes were expressed under the control of their native promoters and terminators. The strains overexpressing PPP genes were cultivated in liquid SC-ura medium containing 1.3% (v/v) or 1.4% isobutanol, or 8% Ethanol at 30°C for 24 h. Error bars represent the SEM of six independent experiments. A two-tailed student's *t*-test was used to assess the statistical significance of the difference between cell growths of control and PPP gene-overexpressing strains in the presence of isobutanol; *$p < 0.001$, **$p < 0.0001$.

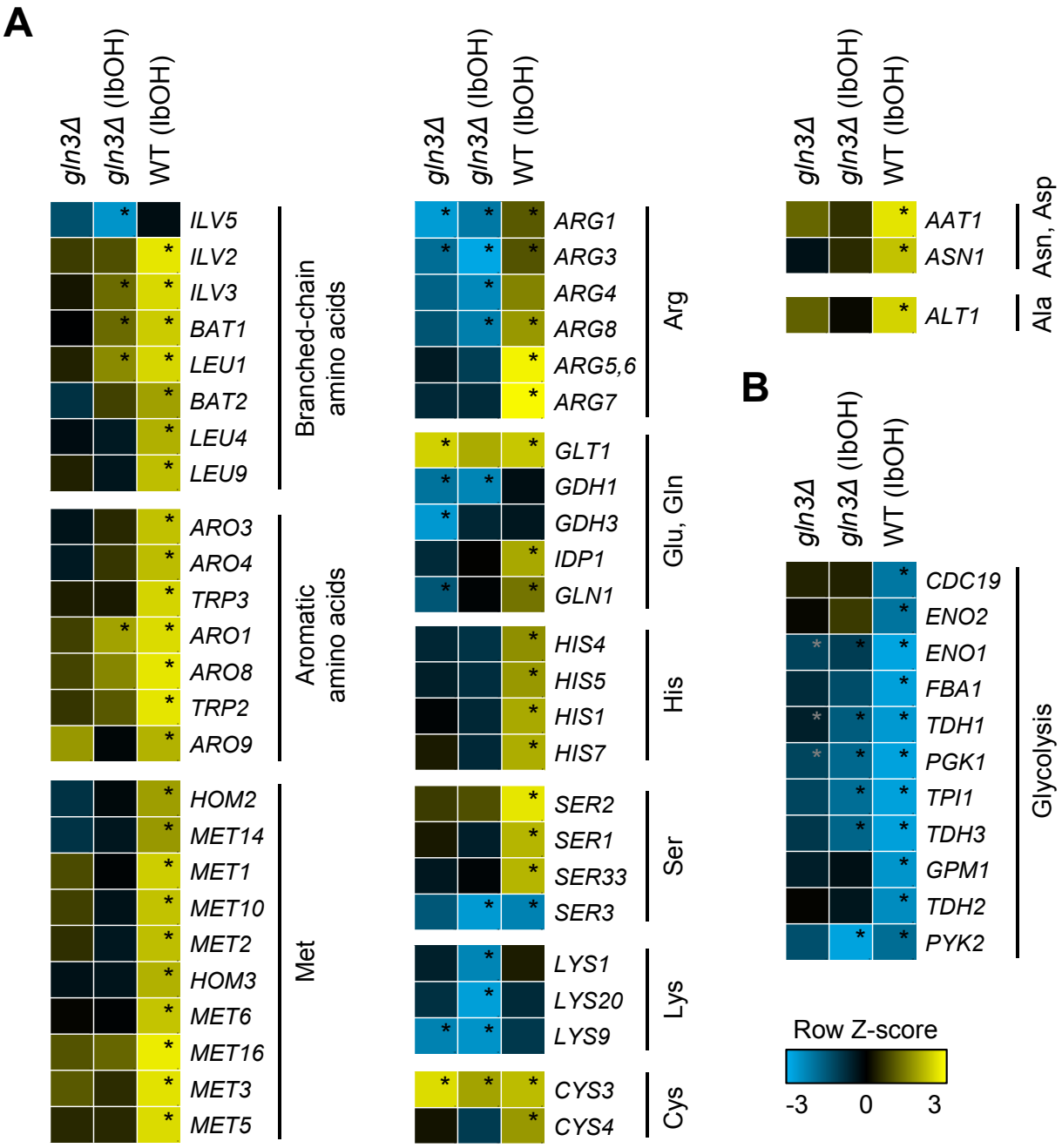


**Figure S6. Heat maps showing expression profiles of genes involved in amino acid biosynthesis and glycolysis (related to Figure 4).** Expression levels of genes involved in amino acid biosynthesis (A) and glycolysis (B) are shown as row Z-score that is the number of standard deviations away from $\log_2$FPKM of the wild type strain grown without isobutanol. Genes in each category were hierarchically clustered with row Z-scores. The statistical significance of the difference from FPKM in the wild type strain grown without isobutanol is shown; *$p < 0.05$.

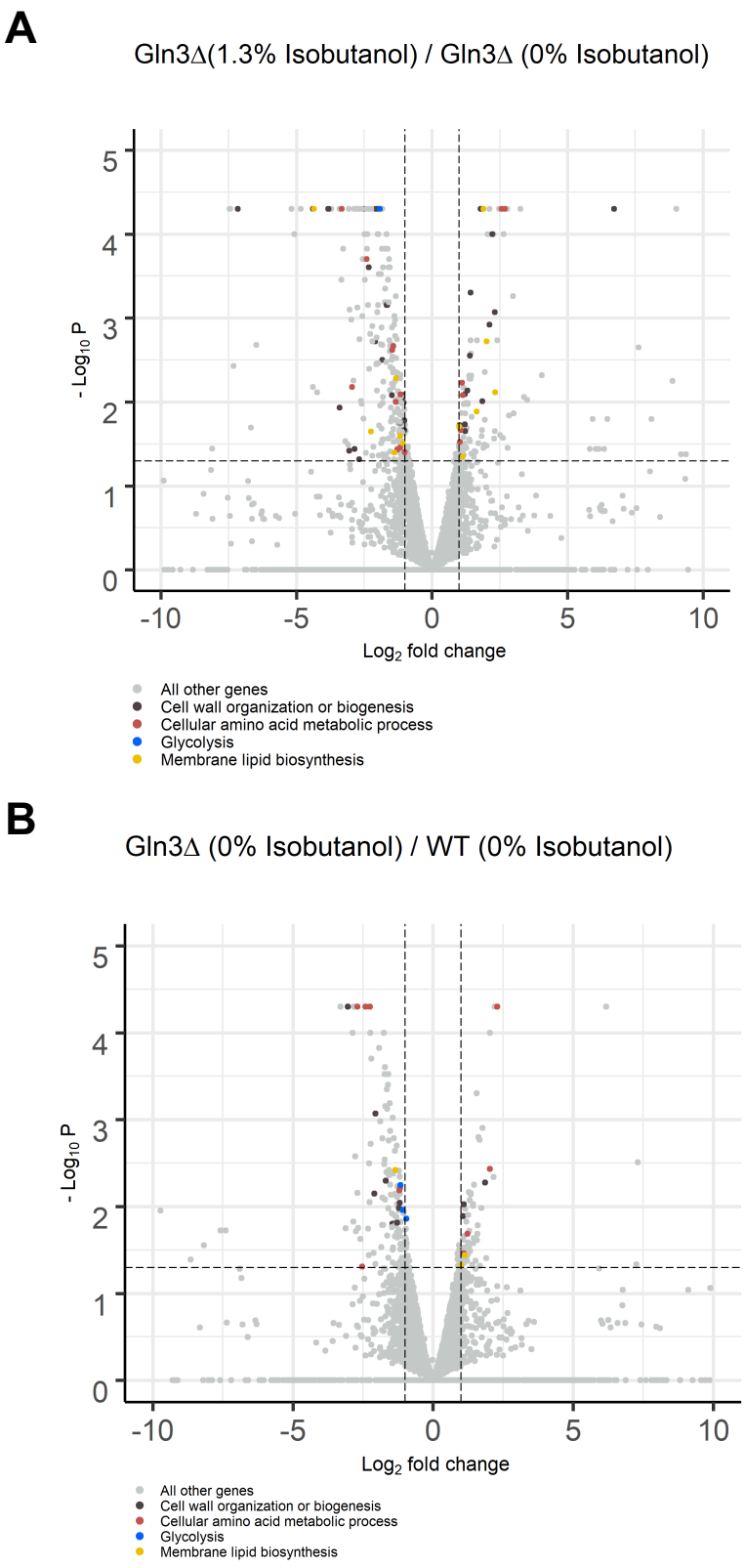


**Figure S7. Volcano plots representing differentially expressed genes (related to Figure 5).** (A) Comparison of the transcriptomic profiles between the *gln3Δ* strain grown with 1.3% (v/v) isobutanol and the *gln3Δ* strain grown without isobutanol. (B) Comparison of the transcriptomic profiles between the *gln3Δ* strain grown without isobutanol and the wild type strain grown without isobutanol. Each dot represents one gene whose position is determined by the average $\log_2$ fold change and negative $\log_{10}$ *p*-value from two independent experiments. The differentially expressed genes are identified on the basis of $|\text{Log}_2$ fold change$| > 1$ and *p*-value $< 0.05$. Genes labeled as involved in "glycolysis" are one of the following: *CDC19, ENO1, ENO2, FBA1, GPM1, PGK1, PYK2, TPI1, TDH1, TDH2, TDH3.* Genes labeled as involved in "membrane lipid biosynthesis" are one of the following: *ACP1, ARE1, AYR1, CHO1, DPP1, EEB1, ELO1, ERG3, ERG4, FAS1, GPC1, HES1, HFD1, HMG2, ICT1, INO1, INP54, LAC1, LSB6, MCR1, OLE1, OPI3, ORM2, PDR16, PLB2, SCS2, SEC59, TAM41, TCB3, TGL2, TSC10, YDC1, YDR018C, YEH1.*

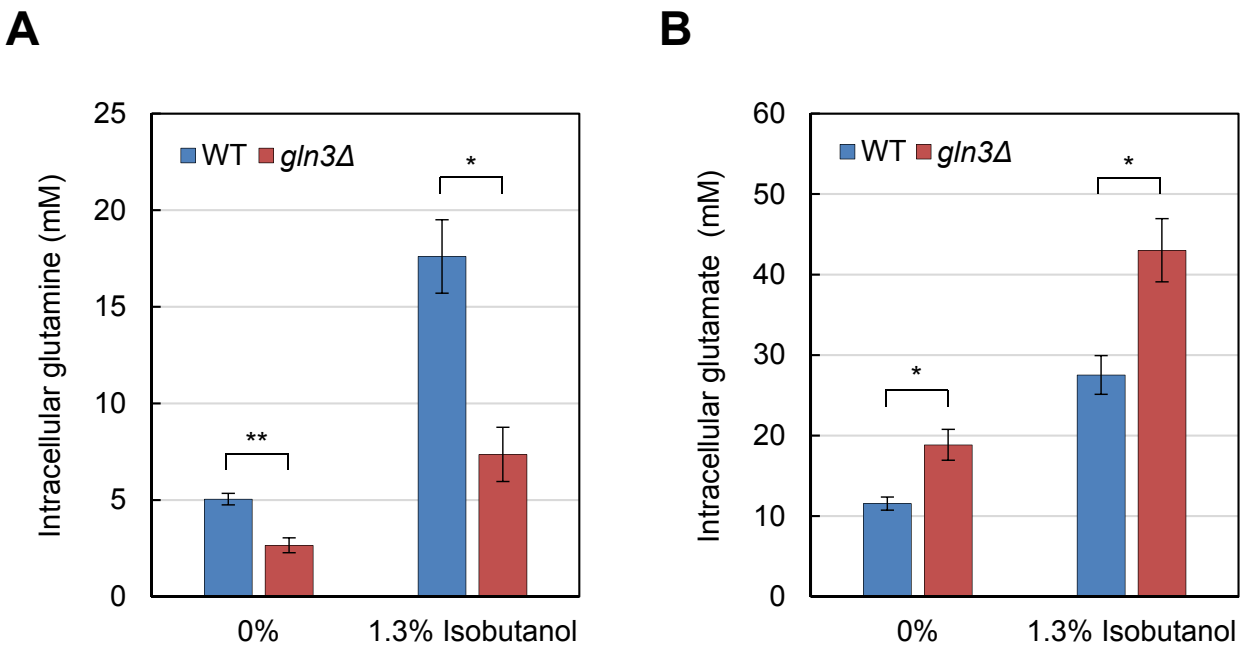


**Figure S8. Intracellular concentrations of glutamine and glutamate in different conditions (related to Figure 4).** Wild type and *gln3Δ* strains were grown in SC medium with or without 1.3% (v/v) isobutanol at 30°C for 12 h. The concentrations of glutamine (A) and glutamate (B) in the metabolites extracted from cells were measured by multiple reaction monitoring (MRM) mode using LC-MS/MS. A two-tailed student's *t*-test was used to assess the statistical significance of the difference between the amino acid concentrations in wild type and *gln3Δ* strains; $*p < 0.01$, $**p < 0.001$. Error bars represent the SEM of six independent experiments.

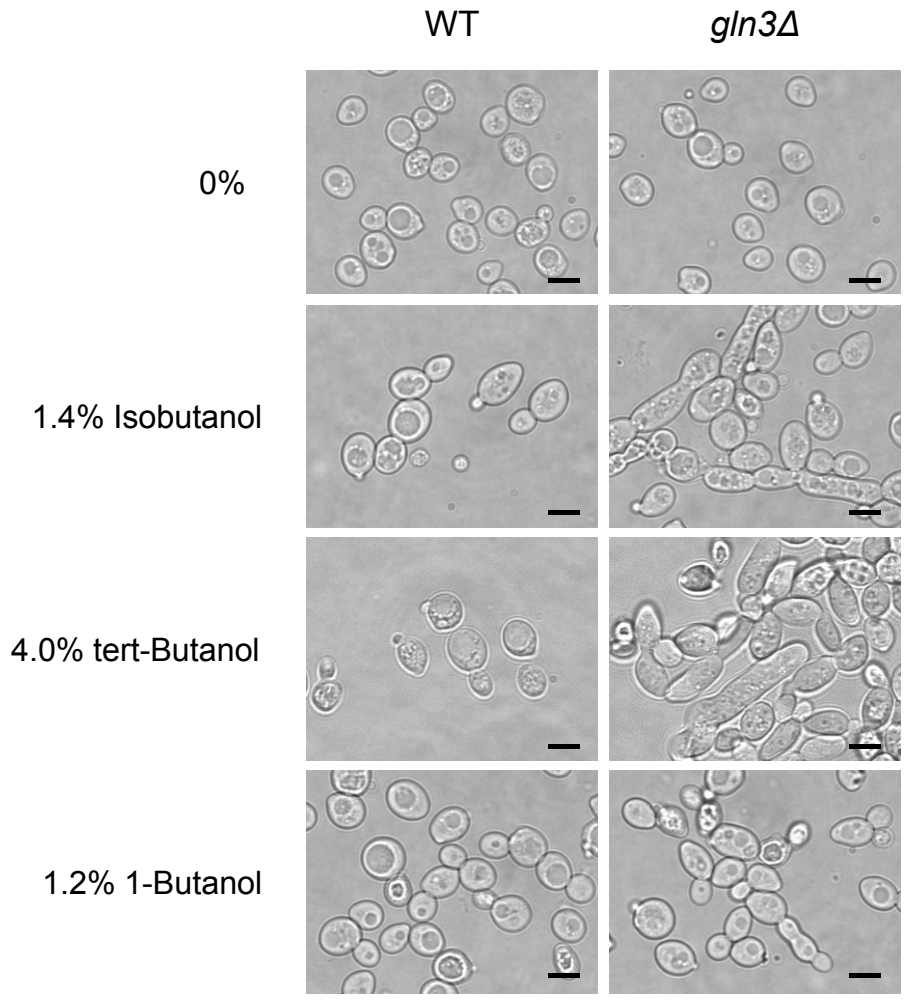


**Figure S9. Morphologies of yeast deletion strains grown with alcohols (related to Figures 2 and 4).** Wild type and *gln3Δ* strains were cultivated in liquid SC medium containing 1.4% (v/v) isobutanol, 4.0% tert-butanol, or 1.2% 1-butanol at 30°C for 24 h. Scale bars indicate 5 μm.

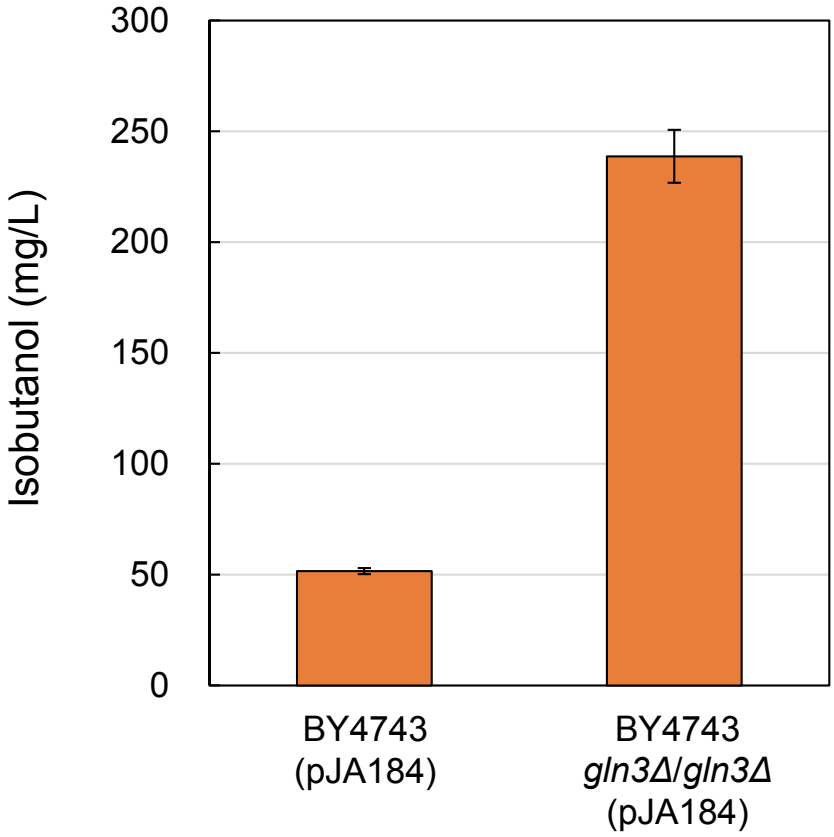


**Figure S10. Isobutanol production by isobutanol-tolerant strain with *GLN3* deletion in inhouse-prepared SC-Ura medium (related to Figure 6).** Isobutanol production of the homozygous BY4743 *gln3Δ*/*gln3Δ* strain in inhouse-prepared SC-Ura medium (Table S8) compared to wild type BY4743, harboring a 2μ plasmid overexpressing the five enzymes responsible for converting pyruvate to isobutanol in their natural locations (pJA184). Error bars represent the SEM of four independent experiments.

**Table S1. Tolerance factors of the wild type BY4741 strain in various concentrations of isobutanol and ethanol (related to Figure 1).** $OD_{600}$ values for 0% and 1.4% isobutanol are shown as red point in Figure 1A.

| Isobutanol (%) | Tolerance factors of the wild type BY4741 |
|---|---|
| 0.6 | 0.92 |
| 1.2 | 0.72 |
| 1.4 | 0.38 |
| 1.5 | 0.19 |
| 1.6 | 0.11 |
| **Ethanol (%)** | **Tolerance factors of the wild type BY4741** |
| 8.0 | 0.31 |

**Table S3. Genes whose deletion leads to sensitivity to isobutanol in the second screen (related to Figure 1B).** Gene deletions in hypersensitive strains (TF < 0.5 in 0.6% isobutanol or TF < 0.1 in 1.2% isobutanol) are highlighted in gray.

| Systematic name | Common name | Tolerance factor in 0.6% isobutanol | Tolerance factor in 1.2% isobutanol | Tolerance factor in 8% EtOH |
|---|---|---|---|---|
| YHR183W | *GND1* | 0.040 | 0.057 | 0.309 |
| YNL241C | *ZWF1* | 0.090 | 0.062 | 0.344 |
| YCR024C | *SLM5* | 0.105 | 0.044 | 0.036 |
| YCR028C | *FEN2* | 0.130 | 0.159 | 0.252 |
| YOR332W | *VMA4* | 0.174 | 0.042 | 0.158 |
| YBR171W | *SEC66* | 0.205 | 0.104 | 0.123 |
| YNL064C | *YDJ1* | 0.206 | 0.102 | 0.095 |
| YER122C | *GLO3* | 0.228 | 0.049 | 0.028 |
| YLR240W | *VPS34* | 0.231 | 0.034 | 0.021 |
| YLR304C | *ACO1* | 0.316 | 0.034 | 0.015 |
| YOR036W | *PEP12* | 0.326 | 0.069 | 0.119 |
| YDR323C | *PEP7* | 0.370 | 0.040 | 0.015 |
| YDR495C | *VPS3* | 0.377 | 0.187 | 0.090 |
| YLR447C | *VMA6* | 0.403 | 0.134 | 0.036 |
| YBR030W | *RKM3* | 0.403 | 0.771 | 0.481 |
| YLR244C | *MAP1* | 0.424 | 0.144 | 0.068 |
| YLL028W | *TPO1* | 0.425 | 0.615 | 0.389 |
| YJL129C | *TRK1* | 0.433 | 0.143 | 0.384 |
| YLR138W | *NHA1* | 0.437 | 0.436 | 0.297 |
| YDR207C | *UME6* | 0.440 | 0.056 | 0.086 |
| YDR316W | *OMS1* | 0.447 | 0.776 | 0.431 |
| YCR034W | *ELO2* | 0.454 | 0.215 | 0.119 |
| YDR315C | *IPK1* | 0.476 | 0.166 | 0.258 |
| YFL023W | *BUD27* | 0.484 | 0.210 | 0.172 |
| YDL160C | *DHH1* | 0.487 | 0.147 | 0.170 |
| YBR018C | *GAL7* | 0.513 | 0.376 | 0.118 |
| YLR182W | *SWI6* | 0.526 | 0.093 | 0.093 |
| YEL027W | *VMA3* | 0.532 | 0.151 | 0.036 |
| YGL173C | *XRN1* | 0.536 | 0.347 | 0.084 |
| YBR173C | *UMP1* | 0.537 | 0.196 | 0.149 |
| YHL011C | *PRS3* | 0.539 | 0.106 | 0.168 |
| YGL168W | *HUR1* | 0.560 | 0.276 | 0.077 |
| YDR417C | - | 0.564 | 0.465 | 0.217 |
| YOR035C | *SHE4* | 0.573 | 0.074 | 0.090 |
| YEL007W | *MIT1* | 0.580 | 0.509 | 0.440 |
| YLR396C | *VPS33* | 0.580 | 0.113 | 0.058 |
| YNL236W | *SIN4* | 0.586 | 0.028 | 0.025 |
| YHR064C | *SSZ1* | 0.586 | 0.263 | 0.101 |
| YBR024W | *SCO2* | 0.586 | 0.841 | 0.412 |
| YGL167C | *PMR1* | 0.587 | 0.295 | 0.079 |
| YBR105C | *VID24* | 0.592 | 0.595 | 0.291 |
| YDL006W | *PTC1* | 0.595 | 0.173 | 0.319 |
| YDR320C | *SWA2* | 0.596 | 0.514 | 0.176 |
| YOR065W | *CYT1* | 0.599 | 0.658 | 0.140 |
| YKL204W | *EAP1* | 0.600 | 0.324 | 0.153 |
| YGL026C | *TRP5* | 0.601 | 0.057 | 0.258 |
| YOR304C-A | *BIL1* | 0.604 | 0.486 | 0.165 |
| YBR179C | *FZO1* | 0.604 | 0.459 | 0.164 |
| YKL212W | *SAC1* | 0.606 | 0.211 | 0.172 |
| YIL076W | *SEC28* | 0.612 | 0.044 | 0.055 |
| YER111C | *SWI4* | 0.618 | 0.119 | 0.103 |

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|---|---|---|---|---|
| YMR142C | *RPL13B* | 0.619 | 0.132 | 0.044 |
| YLR372W | *ELO3* | 0.619 | 0.149 | 0.091 |
| YDL182W | *LYS20* | 0.621 | 0.162 | 0.368 |
| YGL020C | *GET1* | 0.623 | 0.158 | 0.319 |
| YOR290C | *SNF2* | 0.628 | 0.023 | 0.066 |
| YPL060W | *MFM1* | 0.633 | 0.589 | 0.489 |
| YGL072C | - | 0.634 | 0.241 | 0.211 |
| YGR257C | *MTM1* | 0.644 | 0.244 | 0.398 |
| YGR252W | *GCN5* | 0.648 | 0.461 | 0.151 |
| YCL007C | - | 0.650 | 0.148 | 0.091 |
| YBR231C | *SWC5* | 0.652 | 0.568 | 0.302 |
| YLR102C | *APC9* | 0.657 | 0.724 | 0.735 |
| YDR329C | *PEX3* | 0.661 | 0.688 | 0.406 |
| YGR105W | *VMA21* | 0.661 | 0.160 | 0.034 |
| YDR008C | - | 0.661 | 0.045 | 0.183 |
| YNL171C | - | 0.661 | 0.152 | 0.085 |
| YBL098W | *BNA4* | 0.665 | 0.771 | 0.426 |
| YER090W | *TRP2* | 0.669 | 0.038 | 0.189 |
| YDR293C | *SSD1* | 0.670 | 0.119 | 0.091 |
| YJR025C | *BNA1* | 0.682 | 0.112 | 0.059 |
| YGL152C | - | 0.684 | 0.744 | 0.292 |
| YGR285C | *ZUO1* | 0.698 | 0.280 | 0.179 |
| YOR322C | *LDB19* | 0.704 | 0.215 | 0.201 |
| YCL023C | - | 0.705 | 0.586 | 0.543 |
| YOR008C | *SLG1* | 0.708 | 0.505 | 0.141 |
| YDR484W | *VPS52* | 0.713 | 0.156 | 0.118 |
| YKL037W | *AIM26* | 0.714 | 0.347 | 0.143 |
| YBR221C | *PDB1* | 0.720 | 0.312 | 0.187 |
| YCR020W-B | *HTL1* | 0.721 | 0.129 | 0.057 |
| YJL115W | *ASF1* | 0.721 | 0.706 | 0.198 |
| YJL127C | *SPT10* | 0.723 | 0.802 | 0.378 |
| YBR068C | *BAP2* | 0.727 | 0.096 | 0.228 |
| YLR202C | - | 0.730 | 0.041 | 0.004 |
| YOL023W | *IFM1* | 0.731 | 0.191 | 0.131 |
| YJL120W | - | 0.731 | 0.182 | 0.370 |
| YKL118W | - | 0.732 | 0.209 | 0.070 |
| YHR067W | *HTD2* | 0.736 | 0.159 | 0.135 |
| YPL002C | *SNF8* | 0.737 | 0.160 | 0.075 |
| YPR049C | *ATG11* | 0.741 | 0.256 | 0.113 |
| YDL172C | - | 0.744 | 0.129 | 0.363 |
| YNL184C | - | 0.747 | 0.425 | 0.154 |
| YBR003W | *COQ1* | 0.748 | 0.687 | 0.244 |
| YDR127W | - | 0.748 | 0.022 | 0.035 |
| YHR026W | *VMA16* | 0.753 | 0.338 | 0.049 |
| YFL001W | *DEG1* | 0.754 | 0.637 | 0.337 |
| YKL211C | *TRP3* | 0.755 | 0.071 | 0.186 |
| YLR047C | *FRE8* | 0.757 | 0.561 | 0.406 |
| YPL054W | *LEE1* | 0.758 | 0.681 | 0.545 |
| YLR358C | - | 0.762 | 0.148 | 0.035 |
| YLR315W | *NKP2* | 0.764 | 0.188 | 0.270 |
| YJL056C | *ZAP1* | 0.774 | 0.106 | 0.232 |
| YLR337C | *VRP1* | 0.775 | 0.442 | 0.137 |
| YMR312W | *ELP6* | 0.780 | 0.335 | 0.181 |
| YDR101C | *ARX1* | 0.782 | 0.642 | 0.278 |
| YML121W | *GTR1* | 0.784 | 0.471 | 0.115 |
| YJL102W | *MEF2* | 0.785 | 0.645 | 0.224 |
| YER047C | *SAP1* | 0.785 | 0.801 | 0.678 |

| | | | | |
|---|---|---|---|---|
| YJR102C | *VPS25* | 0.795 | 0.263 | 0.097 |
| YPL045W | *VPS16* | 0.796 | 0.048 | 0.021 |
| YJL189W | *RPL39* | 0.800 | 0.336 | 0.203 |
| YGR135W | *PRE9* | 0.803 | 0.550 | 0.282 |
| YKL119C | *VPH2* | 0.809 | 0.291 | 0.070 |
| YBR006W | *UGA2* | 0.816 | 0.826 | 0.519 |
| YJL183W | *MNN11* | 0.816 | 0.122 | 0.084 |
| YBR036C | *CSG2* | 0.819 | 0.120 | 0.070 |
| YLR399C | *BDF1* | 0.824 | 0.291 | 0.091 |
| YNL025C | *SSN8* | 0.827 | 0.371 | 0.069 |
| YNL055C | *POR1* | 0.830 | 0.154 | 0.109 |
| YLR233C | *EST1* | 0.831 | 0.159 | 0.097 |
| YER178W | *PDA1* | 0.832 | 0.545 | 0.428 |
| YDR078C | *SHU2* | 0.850 | 0.094 | 0.004 |
| YDL185W | *VMA1* | 0.851 | 0.234 | 0.029 |
| YGL143C | *MRF1* | 0.854 | 0.055 | 0.013 |
| YJL180C | *ATP12* | 0.854 | 0.620 | 0.250 |
| YKL134C | *OCT1* | 0.854 | 0.335 | 0.292 |
| YLR025W | *SNF7* | 0.861 | 0.248 | 0.110 |
| YDR354W | *TRP4* | 0.863 | 0.143 | 0.365 |
| YDR007W | *TRP1* | 0.872 | 0.110 | 0.272 |
| YDL173W | *PAR32* | 0.874 | 0.136 | 0.315 |
| YBR212W | *NGR1* | 0.877 | 0.745 | 0.533 |
| YJL121C | *RPE1* | 0.882 | 0.080 | 0.287 |
| YLR200W | *YKE2* | 0.892 | 0.694 | 0.207 |
| YDL116W | *NUP84* | 0.895 | 0.551 | 0.328 |
| YLR382C | *NAM2* | 0.895 | 0.483 | 0.190 |
| YJL036W | *SNX4* | 0.896 | 0.351 | 0.187 |
| YDR027C | *VPS54* | 0.897 | 0.103 | 0.090 |
| YPR160W | *GPH1* | 0.898 | 0.382 | 0.088 |
| YOR211C | *MGM1* | 0.899 | 0.499 | 0.201 |
| YDR418W | *RPL12B* | 0.900 | 0.357 | 0.192 |
| YDR018C | - | 0.904 | 0.647 | 0.031 |
| YJL176C | *SWI3* | 0.906 | 0.107 | 0.096 |
| YDL118W | - | 0.911 | 0.741 | 0.366 |
| YPR060C | *ARO7* | 0.913 | 0.050 | 0.066 |
| YPL040C | *ISM1* | 0.920 | 0.098 | 0.010 |
| YOR198C | *YOR198C* | 0.925 | 0.761 | 0.381 |
| YDR193W | - | 0.929 | 0.769 | 0.425 |
| YOR331C | - | 0.931 | 0.366 | 0.062 |
| YJR105W | *ADO1* | 0.931 | 0.071 | 0.115 |
| YJL095W | *BCK1* | 0.939 | 0.177 | 0.336 |
| YGL148W | *ARO2* | 0.942 | 0.048 | 0.061 |
| YDR058C | *TGL2* | 0.945 | 0.274 | 0.339 |
| YNL315C | *ATP11* | 0.956 | 0.417 | 0.259 |
| YPR074C | *TKL1* | 0.966 | 0.617 | 0.623 |
| YKR001C | *VPS1* | 0.979 | 0.297 | 0.138 |
| YGR163W | *GTR2* | 0.982 | 0.544 | 0.159 |
| YOR221C | *MCT1* | 0.992 | 0.438 | 0.262 |
| YOL006C | *TOP1* | 0.996 | 0.117 | 0.099 |
| YOR070C | *GYP1* | 1.011 | 0.397 | 0.299 |
| YOR150W | *MRPL23* | 1.013 | 0.736 | 0.291 |
| YDR173C | *ARG82* | 1.019 | 0.204 | 0.069 |
| YOL004W | *SIN3* | 1.104 | 0.423 | 0.130 |
| YDR162C | *NBP2* | 1.119 | 0.080 | 0.166 |
| YDR184C | *ATC1* | 1.247 | 0.885 | 0.441 |

**Table S4. Genes whose deletion confers highest tolerance to isobutanol (related to Figure 1C).** Gene deletions in hypertolerant strains (TF > 0.4 in 1.5% isobutanol) are highlighted in gray.

| Systematic name | Common name | Tolerance factor in 1.5% isobutanol | Tolerance factor in 1.6% isobutanol | Tolerance factor in 9% EtOH |
|---|---|---|---|---|
| YER040W | *GLN3* | 0.809 | 0.557 | 0.267 |
| YJR044C | *VPS55* | 0.689 | 0.355 | 0.464 |
| YDR508C | *GNP1* | 0.507 | 0.374 | 0.401 |
| YKL146W | *AVT3* | 0.481 | 0.248 | 0.037 |
| YKR026C | *GCN3* | 0.470 | 0.300 | 0.003 |
| YDR391C | - | 0.402 | 0.317 | 0.408 |
| YLL039C | *UBI4* | 0.391 | 0.251 | 0.128 |
| YGR144W | *THI4* | 0.357 | 0.224 | 0.463 |
| YIR005W | *IST3* | 0.353 | 0.175 | 0.000 |
| YKR052C | *MRS4* | 0.303 | 0.167 | 0.003 |
| YLR278C | - | 0.290 | 0.138 | 0.002 |
| YLR280C | - | 0.286 | 0.159 | 0.002 |
| YOR155C | *ISN1* | 0.281 | 0.128 | 0.257 |
| YDR514C | - | 0.280 | 0.166 | 0.315 |
| YKL147C | - | 0.271 | 0.125 | 0.003 |
| YKL051W | *SFK1* | 0.253 | 0.132 | 0.323 |
| YGR124W | *ASN2* | 0.248 | 0.180 | 0.406 |
| YDR134C | - | 0.242 | 0.131 | 0.337 |
| YDL169C | *UGX2* | 0.231 | 0.118 | 0.305 |
| YIL024C | - | 0.231 | 0.159 | 0.148 |
| YLR250W | *SSP120* | 0.224 | 0.118 | 0.002 |
| YGR016W | - | 0.222 | 0.142 | 0.330 |
| YLR236C | - | 0.216 | 0.104 | 0.001 |
| YLR279W | - | 0.213 | 0.105 | 0.002 |
| YIL085C | *KTR7* | 0.211 | 0.095 | 0.001 |
| YJL201W | *ECM25* | 0.207 | 0.122 | 0.297 |
| YPL194W | *DDC1* | 0.201 | 0.132 | 0.171 |
| YLR225C | - | 0.188 | 0.230 | 0.397 |
| YPL197C | - | 0.180 | 0.127 | 0.163 |
| YLR445W | *GMC2* | 0.170 | 0.100 | 0.003 |
| YLR134W | *PDC5* | 0.165 | 0.113 | 0.271 |
| YDR421W | *ARO80* | 0.158 | 0.178 | 0.375 |
| YIL088C | *AVT7* | 0.154 | 0.124 | 0.159 |
| YDL214C | *PRR2* | 0.151 | 0.079 | 0.192 |
| YKL072W | *STB6* | 0.150 | 0.090 | 0.194 |
| YML054C | *CYB2* | 0.140 | 0.087 | 0.219 |

**Table S5. Gene ontology enrichment analysis of the 164 genes whose deletion causes the highest isobutanol sensitivity (related to Figure 1)**

| GO term | Cluster frequency | Background frequency | P-value |
|---|---|---|---|
| Aromatic amino acid family biosynthetic process | 7 (4.3%) | 9 (0.1%) | 7.78E-08 |
| Single-organism cellular process | 98 (59.8%) | 2626 (36.7%) | 8.06E-07 |
| Aromatic amino acid family metabolic process | 9 (5.5%) | 24 (0.3%) | 1.04E-06 |
| Single-organism process | 107 (65.2%) | 3031 (42.3%) | 1.39E-06 |
| Tryptophan biosynthetic process | 5 (3.0%) | 5 (0.1%) | 4.58E-06 |
| Indole-containing compound biosynthetic process | 5 (3.0%) | 5 (0.1%) | 4.58E-06 |
| Indolalkylamine biosynthetic process | 5 (3.0%) | 5 (0.1%) | 4.58E-06 |
| Indole-containing compound metabolic process | 7 (4.3%) | 14 (0.2%) | 6.73E-06 |
| Tryptophan metabolic process | 7 (4.3%) | 14 (0.2%) | 6.73E-06 |
| Indolalkylamine metabolic process | 7 (4.3%) | 14 (0.2%) | 6.73E-06 |
| Vacuolar transport | 19 (11.6%) | 171 (2.4%) | 7.24E-06 |
| Cellular monovalent inorganic cation homeostasis | 9 (5.5%) | 39 (0.5%) | 0.00013 |
| Cellular chemical homeostasis | 16 (9.8%) | 148 (2.1%) | 0.00017 |
| Cellular cation homeostasis | 14 (8.5%) | 115 (1.6%) | 0.00023 |
| Cellular biogenic amine metabolic process | 7 (4.3%) | 22 (0.3%) | 0.00029 |
| Monovalent inorganic cation homeostasis | 9 (5.5%) | 43 (0.6%) | 0.00031 |
| Cellular homeostasis | 17 (10.4%) | 177 (2.5%) | 0.00039 |
| Cation homeostasis | 14 (8.5%) | 123 (1.7%) | 0.00054 |
| Cellular biogenic amine biosynthetic process | 5 (3.0%) | 9 (0.1%) | 0.00054 |
| Amine biosynthetic process | 5 (3.0%) | 9 (0.1%) | 0.00054 |
| Cellular amine metabolic process | 8 (4.9%) | 36 (0.5%) | 0.00086 |
| Amine metabolic process | 8 (4.9%) | 36 (0.5%) | 0.00086 |
| Cellular ion homeostasis | 14 (8.5%) | 128 (1.8%) | 0.00087 |
| Small molecule metabolic process | 36 (22.0%) | 684 (9.5%) | 0.00098 |
| Chemical homeostasis | 16 (9.8%) | 170 (2.4%) | 0.00112 |
| pH reduction | 7 (4.3%) | 27 (0.4%) | 0.00136 |
| Intracellular pH reduction | 7 (4.3%) | 27 (0.4%) | 0.00136 |
| Vacuolar acidification | 7 (4.3%) | 27 (0.4%) | 0.00136 |
| Biological regulation | 63 (38.4%) | 1604 (22.4%) | 0.00159 |
| Autophagy | 15 (9.1%) | 155 (2.2%) | 0.00172 |
| Inorganic ion homeostasis | 13 (7.9%) | 118 (1.6%) | 0.00198 |
| Ion homeostasis | 14 (8.5%) | 138 (1.9%) | 0.00217 |
| Regulation of cellular pH | 7 (4.3%) | 29 (0.4%) | 0.00229 |
| Regulation of intracellular pH | 7 (4.3%) | 29 (0.4%) | 0.00229 |
| Protein localization to vacuole | 13 (7.9%) | 120 (1.7%) | 0.00239 |
| Carboxylic acid metabolic process | 23 (14.0%) | 351 (4.9%) | 0.00331 |
| Regulation of pH | 7 (4.3%) | 32 (0.4%) | 0.00466 |
| Oxoacid metabolic process | 23 (14.0%) | 365 (5.1%) | 0.00635 |
| Organic acid metabolic process | 23 (14.0%) | 365 (5.1%) | 0.00664 |
| Cellular component organization | 68 (41.5%) | 1867 (26.1%) | 0.00781 |

**Table S8. Compositions of synthetic complete (SC) media other than glucose (related to STAR Methods)**

| Components | Final concentration in commercial medium (mg/L) | Final concentration in medium made inhouse (mg/L) |
|---|---|---|
| Adenine | 18 | 95 |
| *p*-Aminobenzoic acid | 8 | 9.5 |
| Ammonium sulfate | 5000 | 5000 |
| Alanine | 76 | 95 |
| Arginine | 76 | 95 |
| Asparagine | 76 | 95 |
| Aspartic acid | 76 | 95 |
| Cysteine | 76 | 95 |
| Glutamic acid | 76 | 95 |
| Glutamine | 76 | 95 |
| Glycine | 76 | 95 |
| Histidine | 76 | 95 |
| Inositol | 76 | 36 |
| Isoleucine | 76 | 95 |
| Leucine | 380 | 190 |
| Lysine | 76 | 95 |
| Methionine | 76 | 95 |
| Phenylalanine | 76 | 95 |
| Proline | 76 | 95 |
| Serine | 76 | 95 |
| Threonine | 76 | 95 |
| Tryptophan | 76 | 95 |
| Tyrosine | 76 | 95 |
| Uracil | 76 | 95 |
| Valine | 76 | 95 |
| Yeast nitrogen base without amino acids | 1700 | 1500 |

**Table S9. Yeast strains used in this study (related to STAR Methods)**

| Strain | Genotype | Source |
|---|---|---|
| BY4741 | S288C *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | Euroscarf: Y00000 |
| BY4743 | S288C *MAT***a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0* | Euroscarf: Y20000 |
| CEN.PK2-1C | *MAT***a** *his3Δ1 leu2-3,112 ura3-52 trp1-289 MAL2-8$^c$ SUC2* | Euroscarf: 30000A |
| SEY6210 | *MAT*α *leu2-3,112 ura3-52 his3-Δ200 trp1-Δ 901 suc2-Δ9 lys2-801 GAL* | ATCC: 96099 |
| BY4741 *aco1Δ* | BY4741 *aco1Δ::kanMX4* | Euroscarf: Y05212 |
| BY4741 *avt3Δ* | BY4741 *avt3Δ::kanMX4* | Euroscarf: Y04996 |
| BY4741 *dhh1Δ* | BY4741 *dhh1Δ::kanMX4* | Euroscarf: Y03858 |
| BY4741 *elo2Δ* | BY4741 *elo2Δ::kanMX4* | Euroscarf: Y05763 |
| BY4741 *gcn3Δ* | BY4741 *gcn3Δ::kanMX4* | Euroscarf: Y05097 |
| BY4741 *gln3Δ* | BY4741 *gln3Δ::kanMX4* | Euroscarf: Y00173 |
| BY4741 *glo3Δ* | BY4741 *glo3Δ::kanMX4* | Euroscarf: Y06121 |
| BY4741 *gnd1Δ* | BY4741 *gnd1Δ::kanMX4* | Euroscarf: Y02877 |
| BY4741 *ipk1Δ* | BY4741 *ipk1Δ::kanMX4* | Euroscarf: Y07747 |
| BY4741 *map1Δ* | BY4741 *map1Δ::kanMX4* | Euroscarf: Y05153 |
| BY4741 *nha1Δ* | BY4741 *nha1Δ::kanMX4* | Euroscarf: Y04095 |
| BY4741 *pep12Δ* | BY4741 *pep12Δ::kanMX4* | Euroscarf: Y01812 |
| BY4741 *rpe1Δ* | BY4741 *rpe1Δ::kanMX4* | Euroscarf: Y01305 |
| BY4741 *sec28Δ* | BY4741 *sec28Δ::kanMX4* | Euroscarf: Y01469 |
| BY4741 *sec66Δ* | BY4741 *sec66Δ::kanMX4* | Euroscarf: Y03311 |
| BY4741 *sol3Δ* | BY4741 *sol3Δ::kanMX4* | Euroscarf: Y02857 |
| BY4741 *swi6Δ* | BY4741 *swi6Δ::kanMX4* | Euroscarf: Y04131 |
| BY4741 *tal1Δ* | BY4741 *tal1Δ::kanMX4* | Euroscarf: Y05263 |
| BY4741 *tkl1Δ* | BY4741 *tkl1Δ::kanMX4* | Euroscarf: Y05493 |
| BY4741 *trp1Δ* | BY4741 *trp1Δ::kanMX4* | Euroscarf: Y07202 |
| BY4741 *trp2Δ* | BY4741 *trp2Δ::kanMX4* | Euroscarf: Y06395 |
| BY4741 *ume6Δ* | BY4741 *ume6Δ::kanMX4* | Euroscarf: Y03566 |
| BY4741 *vma4Δ* | BY4741 *vma4Δ::kanMX4* | Euroscarf: Y01629 |
| BY4741 *vma6Δ* | BY4741 *vma6Δ::kanMX4* | Euroscarf: Y06051 |
| BY4741 *vps34Δ* | BY4741 *vps34Δ::kanMX4* | Euroscarf: Y05149 |
| BY4741 *vps3Δ* | BY4741 *vps3Δ::kanMX4* | Euroscarf: Y04329 |
| BY4741 *vps55Δ* | BY4741 *vps55Δ::kanMX4* | Euroscarf: Y06842 |
| BY4741 *ydj1Δ* | BY4741 *ydj1Δ::kanMX4* | Euroscarf: Y03012 |
| BY4741 *ydr391cΔ* | BY4741 *ydr391cΔ::kanMX4* | Euroscarf: Y04227 |
| BY4741 *ynl170wΔ* | BY4741 *ynl170wΔ::kanMX4* | Euroscarf: Y02041 |
| BY4741 *zwf1Δ* | BY4741 *zwf1Δ::kanMX4* | Euroscarf: Y01971 |
| BY4742 *gnd1Δ* | BY4742 *gnd1Δ::kanMX4* | Euroscarf: Y12877 |
| BY4742 *zwf1Δ* | BY4742 *zwf1Δ::kanMX4* | Euroscarf: Y11971 |
| BY4743 *gln3Δ/gln3Δ* | BY4743 *gln3Δ::kanMX4/gln3Δ::kanMX4* | Euroscarf: Y30173 |
| CEN.PK2-1C *TRP1* | CEN.PK2-1C *TRP1* | This study |
| CEN.PK2-1C *TRP1 gln3Δ* | CEN.PK2-1C *TRP1 gln3Δ::kanMX4* | This study |
| CEN.PK2-1C *TRP1 gnd1Δ* | CEN.PK2-1C *TRP1 gnd1Δ::kanMX4* | This study |
| CEN.PK2-1C *TRP1 zwf1Δ* | CEN.PK2-1C *TRP1 zwf1Δ::kanMX4* | This study |

| | | |
|---|---|---|
| SEY6210 *TRP1* | SEY6210 *TRP1* | This study |
| BY4741 (pRS426) | BY4741 pRS426 | This study |
| BY4741 (*GND1*) | BY4741 pXP684-GND1 | This study |
| BY4741 (*GND2*) | BY4741 pXP684-GND2 | This study |
| BY4741 (*RPE1*) | BY4741 pXP684-RPE1 | This study |
| BY4741 (*SOL3*) | BY4741 pXP684-SOL3 | This study |
| BY4741 (*TAL1*) | BY4741 pXP684-TAL1 | This study |
| BY4741 (*TKL1*) | BY4741 pXP684-TKL1 | This study |
| BY4741 (*TKL2*) | BY4741 pXP684-TKL2 | This study |
| BY4741 (*ZWF1*) | BY4741 pXP684-ZWF1 | This study |
| BY4741 (pJA184) | BY4741 pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |
| BY4741 *gln3Δ* (pRS426) | BY4741 *gln3Δ::lox66-natMX6-lox71* pRS426 | This study |
| BY4741 *gln3Δ* (pJA184) | BY4741 *gln3Δ::lox66-natMX6-lox71* pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |
| BY4741 *ald6Δ* (pRS426) | BY4741 *ald6Δ::loxP-kanMX4-loxP* pRS426 | This study |
| BY4741 *ald6Δ* (pJA184) | BY4741 *ald6Δ::loxP-kanMX4-loxP* pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |
| BY4741 *ald6Δ gln3Δ* (pRS426) | BY4741 *gln3Δ::lox66-natMX6-lox71 ald6Δ::loxP-kanMX4-loxP* pRS426 | This study |
| BY4741 *ald6Δ gln3Δ* (pJA184) | BY4741 *gln3Δ::lox66-natMX6-lox71 ald6Δ::loxP-kanMX4-loxP* pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |
| BY4743 (pRS426) | BY4743 pRS426 | This study |
| BY4743 *gln3Δ/gln3Δ* (pRS426) | BY4743 *gln3Δ::kanMX4/gln3Δ::kanMX4* pRS426 | This study |
| BY4743 (pJA184) | BY4743 pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |
| BY4743 *gln3Δ/gln3Δ* (pJA184) | BY4743 *gln3Δ::kanMX4/gln3Δ::kanMX4* pJA184 (*ILV2*, *ILV3*, *ILV5*, *LlKivD*, *ADH7*) | This study |

**Table S10. Plasmids used in this study (related to STAR Methods)**

| Plasmid | Description | Source |
|---|---|---|
| pXP684-GND1 | Overexpression of *GND1* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-GND2 | Overexpression of *GND2* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-RPE1 | Overexpression of *RPE1* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-SOL3 | Overexpression of *SOL3* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-TAL1 | Overexpression of *TAL1* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-TKL1 | Overexpression of *TKL1* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-TKL2 | Overexpression of *TKL2* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pXP684-ZWF1 | Overexpression of *ZWF1* by 2μ *URA3* plasmid containing the endogenous promoter, ORF, and terminator sequence | Huang et al., 2013 |
| pRS426 | Empty plasmid with 2μ and *URA3* | ATCC: 96099 |
| pJA184 | Isobutanol production by 2μ *URA3* plasmid containing $P_{TDH3}$-*ILV2-HA*-$T_{ADH1}$, $P_{PGK1}$-*ILV3-His*-$T_{CYC1}$, $P_{TEF1}$-*ADH7-Myc*-$T_{ACT1}$, [$P_{TEF1}$-*ILV5*-*Myc*-$T_{ACT1}$, $P_{TDH3}$-*LlKivD*-*HA*-$T_{ADH1}$] | Avalos et al., 2013 |
| pYZ84 | Plasmid containing the *lox66-natMX6-lox71* deletion cassette | Hammer and Avalos, 2017 |
| pUG6 | Plasmid containing the *loxP-kanMX4-loxP* deletion cassette | Gueldener et al., 2002 |

* Brackets indicate reverse compliments.

**Table S11. Primers used in this study (related to STAR Methods)**

| Primer | Sequence (5'–3') | Target region or description |
|---|---|---|
| TRP1-Pro-F | ACACTGAGTAATGGTAGTTATAAGAAAGAG | $P_{TRP1}$-*TRP1*-$T_{TRP1}$ |
| TRP1-Term-R | TGGTGTTTATGCAAAGAAACCACTGTGTTT | |
| GLN3-F | TCTTGCAAGACAGAGAAAGATGTTC | 5' Flanking sequence of *GLN3*-*KanMX4*-3' flanking sequence of *GLN3* |
| GLN3-D | AAACAAATAATACCAATGCTCAGGA | |
| GND1-A | TAAATCACCTGCTACCTCTCTGTTC | 5' Flanking sequence of *GND1*-*KanMX4*-3' flanking sequence of *GND1* |
| GND1-D | TTTTCTGACTTCATGATTTTGTGTC | |
| ZWF1-A | ATTATTAATGTGGGATTTTTGGCTC | 5' Flanking sequence of *ZWF1*-*KanMX4*-3' flanking sequence of *ZWF1* |
| ZWF1-D | TCAATGATAAGTACAAGTCCAATCG | |
| ALD6-KO-F | TCTTGTTTTATAGAAGAAAAAACATCAAGAAACATCTTTAACATACACAAACACATACTATCAGAATACATACGCTGCAGGTCGACAACC | 5' Flanking sequence of *ALD6*-*KanMX4*-3' flanking sequence of *ALD6* |
| ALD6-KO-R | GACGTAAGACCAAGTAAGTTTATATGAAAGTATTTTGTGTATATGACGGAAAGAAATGCAGGTTGGTACACTAGTGGATCTGATATCACC | |
| GLN3-KO-F | ATAACAGAGTGTGTAAGAAAGAGAGACGAGAGAGAGCACAGGGCCCCCTTTTCCCCCACCAACAAACAATACGCTGCAGGTCGACAACC | 5' Flanking sequence of *GLN3*-*NatMX6*-3' flanking sequence of *GLN3* |
| GLN3-KO-R | GAAAATCTATCAATGCAACCGTTCAGTAATTATTAACATAATAAGAATAATGATAATGATAATACGCGGCTAGTGGATFTGATATCACC | |
| GLN3-F2 | TTTGCTCTATTACCCGGCGGACAGG | Forward primer annealing upstream of the introduced DNA fragment for *GLN3* deletion |
| GND1-A2 | CCCTTCTACATAACTCCATGCATGC | Forward primer annealing upstream of the introduced DNA fragment for *GND1* deletion |
| ZWF1-A2 | TGCTAAAAGCCCGGTTTCGGCTCGG | Forward primer annealing upstream of the introduced DNA fragment for *ZWF1* deletion |
| ALD6-F | GGGATTCAAGACAAGCAACCTTGTTAGTCA | Forward primer annealing upstream of the introduced DNA fragment for *ALD6* deletion |
| Jla_oli239 | ATTCGTCGTCGGGGAACACC | Reverse primer annealing within *NatMX6* |
| JW38 | GCACGTCAAGACTGTCAAGG | Reverse primer annealing within *KanMX4* |

**Table S12. Analytical methods to quantify amino acids by LC-MS/MS (related to STAR Methods)**

| Amino acid | Compound abbreviation | MRM transition | Q1 Pre Bias (V) | Q3 Pre Bias (V) | Collision energy (V) | Retention time (min) |
|---|---|---|---|---|---|---|
| L-Glutamic acid | E | 148.1>130.1 | -29 | -2 | -1 | 2.81 |
| L-Glutmaine | Q | 147.1>56.2 | -3 | -2 | -32 | 2.48 |
| L-Glutamic acid-$^{13}C_5$,$^{15}N$ | | 154.1>89.0 | -29 | -17 | -2 | 2.81 |
| L-Glutamine-$^{13}C_5$,$^{15}N_2$ | | 154.1>60.0 | -3 | -2 | -32 | 2.47 |