**Additional file 2**

**Table S2. Yeast strains and plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| Yeast strains  | Description | Source |
| S. cerevisiae strains |  |  |
| BY4742 | MATα HIS3 LEU2 LYS2 URA3 | Research Genetics |
| BY4742/pRS416 | BY4742 (pRS416) | This study  |
| BY4742/IrrE | BY4742 (pRS416-HXT7p-IrrE-TEF1t) | This study  |
| BY4742/I12 | BY4742 (pRS416-HXT7p-IrrEM74T, I103T, S133R, P162S, V204A, V299A, A300V-TEF1t) | This study  |
| BY4742/I24 | BY4742 (pRS416-HXT7p-IrrEA52E, E119V, L160F, R244G -TEF1t) | This study  |
| BY4742/I37 | BY4742 (pRS416-HXT7p-IrrEA52E, L57P, L65P, E119V, L160F, M169V, R244G, E271K and Base 824 Deletion-TEF1t) | This study  |
| M74T | BY4742 (pRS416-HXT7p-IrrEM74T -TEF1t) | This study |
| I103T | BY4742 (pRS416-HXT7p-IrrEI103T-TEF1t) | This study |
| S133R | BY4742 (pRS416-HXT7p-IrrES133R -TEF1t) | This study |
| P162S | BY4742 (pRS416-HXT7p-IrrEP162S -TEF1t) | This study |
| V204A | BY4742 (pRS416-HXT7p-IrrEV204A -TEF1t) | This study |
| V299A | BY4742 (pRS416-HXT7p-IrrEV299A -TEF1t) | This study |
| A300V | BY4742 (pRS416-HXT7p-IrrEA300V-TEF1t) | This study |
| A52E | BY4742 (pRS416-HXT7p-IrrEA52E-TEF1t) | This study |
| E119V | BY4742 (pRS416-HXT7p-IrrEE119VTEF1t) | This study |
| L160F | BY4742 (pRS416-HXT7p-IrrEL160F-TEF1t) | This study |
| R244G | BY4742 (pRS416-HXT7p-IrrER244G-TEF1t) | This study |
| L57P | BY4742 (pRS416-HXT7p-IrrEL57P-TEF1t) | This study |
| L65P | BY4742 (pRS416-HXT7p-IrrEL65P-TEF1t) | This study |
| M169V | BY4742 (pRS416-HXT7p-IrrEM169V-TEF1t) | This study |
| E271K | BY4742 (pRS416-HXT7p-IrrEE271K-TEF1t) | This study |
| Base 824 Deletion | BY4742 (pRS416-HXT7p-IrrEBase 824 Deletion-TEF1t) | This study |

**Table S3 Primers used in this work with endonuclease restriction sites underlined and italicized as essential.**

|  |  |
| --- | --- |
| Primer ID | Sequence (5’-3’) |
| HXT7p\_F | CCCCCCGGGAGAAGGTTTTGGGACGCTC |
| HXT7p\_R | CGGAATTCTTTTTGATTAAAATTAAAAAAACTTTTTG |
| TEF1t\_F | ACGCGTCGACAAATAAGGAGATTGATAAGACTTTTC |
| TEF1t\_R | CCCTCGAGGGCTAACTCTCAACAGACAACAAC |
| IrrE\_F | CGGAATTCATGAAGGATGCTAATGAGAGTAAAT |
| IrrE\_R | ACGCGTCGACTCAACGAGGTGGGAATGCC |
| 74\_F | ATTCTCTGACGGCCGGTGTT |
| 74\_R | AACACCGGCCGTCAGAGAAT |
| 103\_F | TATTCTGACCAACTCTGCGGC |
| 103\_R | GCCGCAGAGTTGGTCAGAATA |
| 133\_F | CCTGCTGAGAGACATCCACG |
| 133\_R | CGTGGATGTCTCTCAGCAGG |
| 162\_F | CCTCATGTCTGAGCCTGTA |
| 162\_R204\_F204\_R | TACAGGCTCAGACATGAGGAAACCCCGGCTCCTGTTATCTACAGATAACAGGAGCCGGGGTTTG |
| 299\_F | GTCTCGTGGTATCGCTGCTG  |
| 299\_R | CAGCAGCGATACCACGAGAC |
| 300\_F | TATCGTTGTTGTTAGCTTTG |
| 300\_R | CAAAGCTAACAACAACGATA |
| 52\_F | AGCGGCGAAGGAGCGTAT |
| 52\_R | ATACGCTCCTTCGCCGCT |
| 119\_F | CTCGCGCATGTAATCGGTC |
| 119\_R | GACCGATTACATGCGCGAG |
| 160\_F | GCAGCGATCTTCATGCC |
| 160\_R | GGCATGAAGATCGCTGC |
| 244\_F | TCTTCTTCTACCGGTGGTG |
| 244\_R | CACCACCGGTAGAAGAAGA |
| 57\_F | TGCGTGACCCGGCGGCAGCGTAC |
| 57\_R | ACGCTGCCGCCGGGTCACGCATAC |
| 65\_F | ACGTTGCGGCGCCCCCAGGT |
| 65\_R | ACCTGGGGGCGCCGCAACGTAC |
| 169\_F | TCGCGGAAGTGCTGGAGCGTTTTG |
| 169\_R | AAACGCTCCAGCACTTCCGC |
| 271\_F | ACCGGCATGAAAGTTCGTGAAG |
| 271\_R | TTCACGAACTTTCATGCCGG |
| 824\_F | TGGAAGTTCGTGAAGATCCTATGTTCCTTTC |
| 824\_R | AGGAACATAGGATCTTCACGAACTTCC |

**Table S4. Upregulated genes related to FAP stress response in the strain BY4742/IrrE.**

|  |  |
| --- | --- |
| Categories  | Genes (Fold Change) |
| DNA repair | RAD52(3.21)、RAD51(2.22)、RAD14(2.19)、PSO2(2.01)、RAD33(2.08) |
| Transcription factors/ activators | YAP1(2.75)、CIN5(2.90)、RGM1(4.75)、RRN11(2.67)、MSS11(2.28)、TAF11(2.23)、HOT1(2.09)、RPI1(2.77)、MSA2(2.90)、HAA1(2.05) |
| membrane proteins  | ATR1(2.88)、LSP1(3.97)、SEG1(2.25)、FMP45(7.20)、YNL194C(5.18)、PST2(2.59)、RTN2(7.41)、TFS1(4.29)、SRC1(3.00)、YME2(2.65)、OM45(2.60)、AIP1(2.49)、HEM15(2.98)、VID24(2.14)、COX14(2.95)、MRL1(2.56)、MCD4(2.19)、YTA12(2.05)、OM14(2.09)、ASI1(2.63)、UIP4(2.33)、TVP18(2.12)、PET111(2.49)、SAM37(2.16)、INP1(2.27)、IMP1(2.03)、LDS1(2.16) |
| transport proteins | AGP1(3.40)、GAP1(3.54)、PNS1(4.51)、TPO3(2.44)、TNA1(2.58)、MUP1(2.74)、MEP2(3.13)、MCH5(2.05)、FET4(2.34)、PTR2(2.23)、UBX2(2.29) |
| Ribosome proteins | MRPL3(2.85)、YML6(2.13)、MRPS17(2.33)、RSM7(2.89)、MRPL33(2.43)、RPL15B(2.19)、MRPS8(3.00)、TMA23(2.08)、MRPL19(2.18)、MRPL51(2.14) |

**Table S5. Upregulated genes related to FAP stress response in the strain BY4742/I24.**

|  |  |
| --- | --- |
| Categories  | Genes (Fold Change) |
| permease | GNP1(3.24)、TPO3(3.12)、GAP1(3.35)、MUP1(3.09)、TPO2(3.97)、AGP1(2.64)、DIP5(2.35)、PUT4(2.23) |
| Translation initiation factor | TIF1(3.93)、TIF4631(2.49)、TIF11(2.42)、TIF3(2.23)、CDC33(2.35)、NIP1(2.54)、RPG1(2.39)、PRT1(2.09)、CLU1(2.46)、RLI1(2.20)、GCN3(2.09) |
| Transcription factors/activators | MSN4(2.71)、MSN2(2.28)、PHD1(2.24)、MCM1(2.33)、RAP1(2.02) 1(2.09)、CLU1(2.46)、RLI1(2.20)、GCN3(2.09) |
| Membrane related  | ELO3(3.42)、PMA1(2.56)、PMP1(3.35) |

**Figure S1**

****

The growth behaviors of strain BY4742/pRS416 and BY4742/IrrE under acetic acid condition.

**Figure S2**

****

Transcriptional profiles of ribosome biogenesis by expressing I24. The strain BY4742/I24 and control strain BY4742/pRS416 were cultured in SC-Ura medium with FAP tolerance. Samples were taken in the middle of the lag phase. Box number exhibits transcriptional change, which is the foldchange of the transcriptional level of the strain BY4742/I24 to that of the control strain BY4742/pRS416. Upregulated genes were highlighted in red.

**Figure S3**



The growth behaviors, glucose consumption and ethanol production of strain BY4742/pRS416, BY4742/IrrE, BY4742/I12, BY4742/I24 and BY4742/I37 at 38 ºC in the presence of 0.8 g/L furfural, 3.0 g/L acetic acid and 0.3 g/L phenol.

**Figure S4**



DEGs in the strain BY4742/IrrE in the presence of 0.8 g/L furfural, 3.0 g/L acetic acid and 0.3 g/L phenol. log2foldchange>1.0 and *p* value<0.05

**Figure S5**



Representation of differentially expressed genes in selected Gene Ontology (GO) categories and KEGG pathways in the strain BY4742/IrrE after exposure to multiple inhibitors (0.8 g/L furfural, 3.0 g/L acetic acid and 0.3 g/L phenol) until the middle of the lag phase. (a) The most enriched GO terms of the upregulated genes. (b) The statistics of pathway enrichment of the upregulated genes. (c) The most enriched GO terms of the downregulated genes. (d) The statistics of pathway enrichment of the downregulated genes.

**Figure S6**

****

Representation of differentially expressed genes in selected Gene Ontology (GO) categories and KEGG pathways in the strain BY4742/I24 after exposure to multiple inhibitors (0.8 g/L furfural, 3.0 g/L acetic acid and 0.3 g/L phenol) until the middle of the lag phase. (a) The most enriched GO terms of the upregulated genes. (b) The statistics of pathway enrichment of the upregulated genes. (c) The most enriched GO terms of the downregulated genes. (d) The statistics of pathway enrichment of the downregulated genes.

**Figure S7**



Plasmid map for plasmid pRS416-HXT7p-IrrE-TEF1t and IrrE library construction