

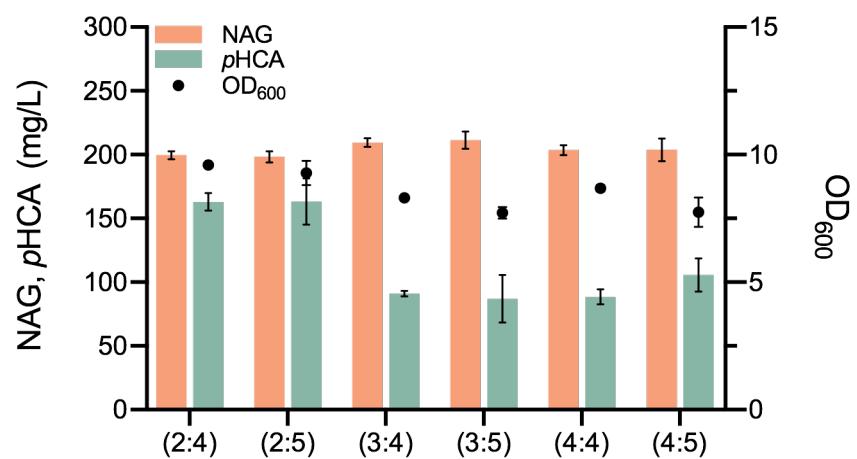
# **Fine-tuning of coumaric acid synthesis to increase naringenin production in yeast**

Jiwei Mao<sup>a</sup>, Marta Tous Mohedano<sup>a</sup>, Xiaowei Li<sup>a</sup>, Quanli Liu<sup>a</sup>, Jens Nielsen<sup>a,b</sup>, Verena Siewers<sup>a</sup>, Yun Chen<sup>a\*</sup>

<sup>a</sup>Department of Biology and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden

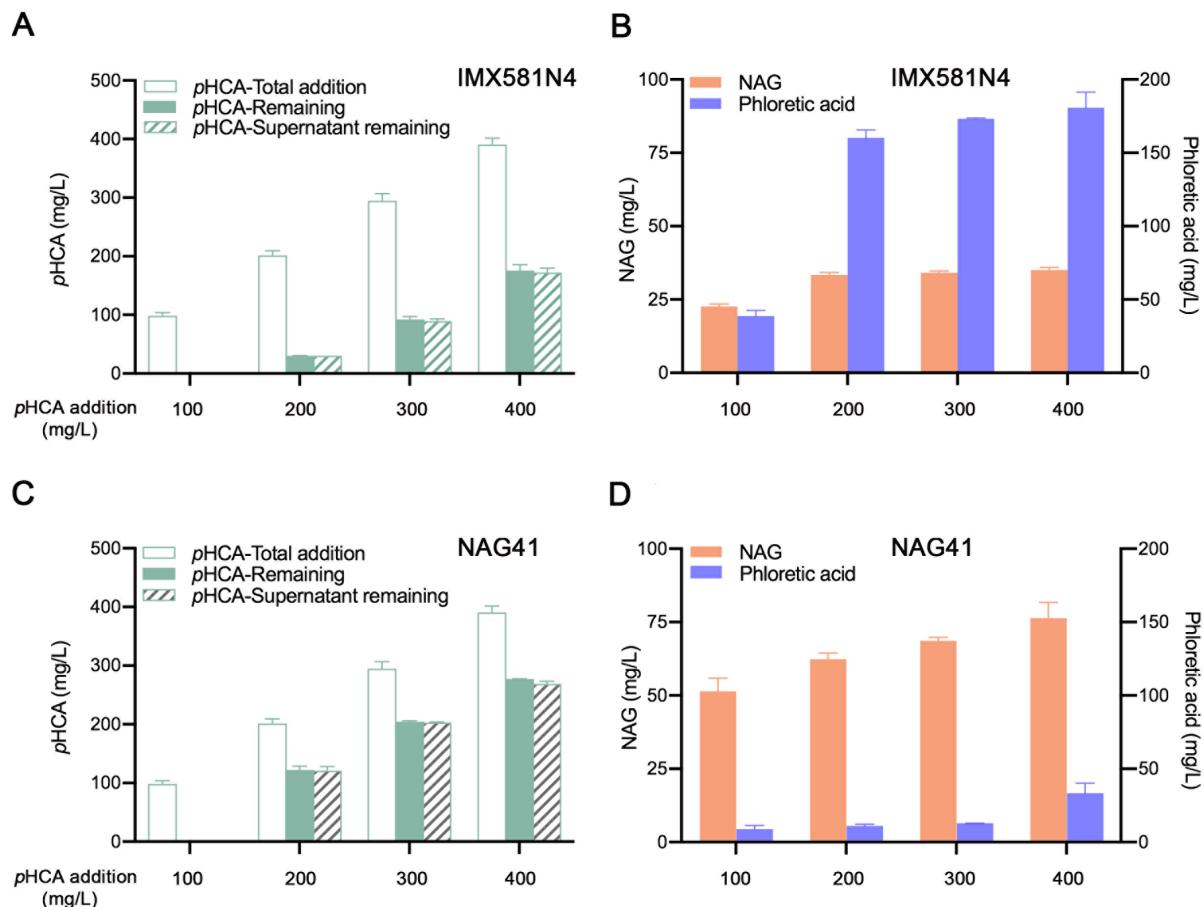
<sup>b</sup>BioInnovation Institute, DK2200 Copenhagen N, Denmark

**Figure S1.**



**Figure S1. Optimization of the downstream enzymes of *p*-coumaric acid for (2S)-naringenin production.** The numbers (n : n) indicate the respective gene copy numbers of 4CL and CHS&CHI. Cells were grown in a defined minimal medium with 30 g/L glucose as the sole carbon source, and cultures were sampled after 96 h of growth for metabolite analysis. All data represent the mean of n = 3 biologically independent samples and error bars show standard deviation.

**Figure S2**



**Figure S2. Production of (2S)-naringenin in strains supplemented with different concentrations of *p*-coumaric acid.** (A) (B) (2S)-naringenin production by the IMX581N4 strain expressing one copy of *4CL* and *CHS&CHI* with the supplementary of different concentrations of *p*-coumaric acid. Cells were grown in a defined minimal medium with 30 g/L glucose as the sole carbon source and with supplemented *p*-coumaric acid (100, 200, 300, 400 mg/L) as precursor. Cultures were sampled after 96 h of growth for metabolite detection. (C) (D) Production of (2S)-naringenin in strain NAG41 supplemented with different concentrations of *p*-coumaric acid. Strain NAG41 harboring three copies of *4CL* and four copies of *CHS&CHI*, simultaneously expressing the *ACC1* mutant and the malonate assimilation pathway (*matBC*). Cells were grown in a defined minimal medium with 30 g/L glucose as the sole carbon source and 2 g/L sodium malonate dibasic, and with supplemented *p*-coumaric acid (100, 200, 300, 400 mg/L) as precursor. Cultures were sampled after 96 h of growth for metabolite analysis. All data represent the mean of  $n \geq 2$  biologically independent samples and error bars show standard deviation.

**Supplementary Table 1. *S. cerevisiae* strains used in this study.**

| Strain ID | Genotype   | Parental strain | Origin    |
|-----------|--|-----------------|-----------|
| IMX581    | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3</i>  |                 | (1)       |
| QL01      | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)</i>   | IMX581          | (2)       |
| NAG01     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-HaCHS-CCW12p)+(tHXT7p-PhCHI-FBA1t)</i>  | QL01            | This work |
| NAG02     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-HaCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>  | QL01            | This work |
| NAG03     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-HaCHS-CCW12p)+(tHXT7p-SmCHI-FBA1t)</i>  | QL01            | This work |
| NAG04     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PhCHI-FBA1t)</i>  | QL01            | This work |
| NAG05     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>  | QL01            | This work |
| NAG06     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-SmCHI-FBA1t)</i>  | QL01            | This work |
| NAG07     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-SmCHS-CCW12p)+(tHXT7p-PhCHI-FBA1t)</i>  | QL01            | This work |
| NAG08     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-SmCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>  | QL01            | This work |
| NAG09     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-SmCHS-CCW12p)+(tHXT7p-SmCHI-FBA1t)</i>  | QL01            | This work |
| NAG10     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>                          | NAG05           | This work |
| NAG11     | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) X-2::( GPM1p-PHA2-CYC1t) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i> | NAG010          | This work |
| NAG1-2    | MAT <sub>a</sub> <i>ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-</i>  | NAG010          | This work |

|        |   |        |           |
|--------|---|--------|-----------|
|        | $CCW12p) + (tHXT7p-PsCHI-FBA)$ XII-5-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA)  |        |           |
| NAG1-3 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA) XI-1-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA)  | NAG1-2 | This work |
| NAG1-4 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA) XI-1-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA) XII-1-:(TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA)                       | NAG1-3 | This work |
| NAG2-1 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t)  | NAG010 | This work |
| NAG2-2 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA)  | NAG10  | This work |
| NAG2-3 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA)  | NAG2-2 | This work |
| NAG2-4 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA) XI-1-:(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA) | NAG2-3 | This work |
| NAG3-1 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t) XII-5-:(CYC1t-At4CL-TPIp)  | NAG2-1 | This work |
| NAG3-2 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t)$ X-3-:(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 <sup>G141S</sup> -TEF1p)+(PGK1p-ARO4 <sup>K229L</sup> -CYC1t) X-4-:(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1-:(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5-:(CYC1t-At4CL-TPIp)  | NAG2-2 | This work |

|           |   |           |           |
|-----------|---|-----------|-----------|
| NAG3-3    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPI1p)</i>  | NAG2-2    | This work |
| NAG3-4    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA)</i>                       | NAG3-3    | This work |
| NAG4-1    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) XII-5::(CYC1t-At4CL-TPI1p) XI-1::(CYC1t-At4CL-TPI1p)</i>   | NAG3-1    | This work |
| NAG4-2    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(CYC1t-At4CL-TPI1p) XI-1::(CYC1t-At4CL-TPI1p)</i>   | NAG3-2    | This work |
| NAG4-3    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPI1p) XI-1::(CYC1t-At4CL-TPI1p)</i>  | NAG3-3    | This work |
| NAG4-4    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPI1p)</i> | NAG3-3    | This work |
| NAG-CHS*2 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(TEF1p-RsCHS-FBA)</i>  | NAG10     | This work |
| NAG-CHS*3 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(TEF1p-RsCHS-FBA) XI-1::(TEF1p-RsCHS-FBA)</i>  | NAG-CHS*2 | This work |

|           |   |           |           |
|-----------|---|-----------|-----------|
| NAG-CHI*2 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-5::(pYX212t-PsCHI-PGKp)</i>   | NAG10     | This work |
| NAG-CHI*3 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-5::(pYX212t-PsCHI-PGKp) XI-1::(pYX212t-PsCHI-PGKp)</i>  | NAG-CHI*2 | This work |
| NAG2-5    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA1t) XI-1::(pYX212t-PsCHI-PGKp) + (TEF1p-RsCHS-FBA1t) XI-3::(TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>   | NAG2-4    | This work |
| NAG3-5    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-1::(TDH3p-A14CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA1t) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA1t) XI-3::(TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i>                       | NAG3-4    | This work |
| NAG4-5    | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA1t) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA1t) + (CYC1t-At4CL-TPI1p) XI-3::(TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t)</i> | NAG4-4    | This work |
| NAG12     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t)</i>   | NAG010    | This work |
| NAG13     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i>   | NAG010    | This work |
| NAG14     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA1t) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i>   | NAG12     | This work |

|       |  |        |           |
|-------|--|--------|-----------|
| NAG37 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XII-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t)</i>   | NAG3-4 | This work |
| NAG38 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XII-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t)</i>   | NAG3-4 | This work |
| NAG39 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)+(TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i>        | NAG37  | This work |
| NAG41 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i>   | NAG39  | This work |
| NAG58 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(GPM1p-AtPAL2-FBA1t)</i>  | NAG39  | This work |
| NAG59 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) + (CYC1t-At4CL-TPI1p) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBAt) XI-2::(TPI1p-ACC1<sup>S659A,S1157A</sup>-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(CCW12p-AtPAL2-FBA1t)</i> | NAG39  | This work |
| NAG60 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7<sup>G141S</sup>-TEF1p)+(PGK1p-ARO4<sup>K229L</sup>-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBAt)</i>  | NAG39  | This work |

|         |   |       |           |
|---------|---|-------|-----------|
|         | <i>FBA1t</i> XII-5::( <i>pYX212t-PsCHI-PGKp</i> ) + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>pYX212t-PsCHI-PGKp</i> ) + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(CCW12_BS2p-AtPAL2-FBA1t)</i>  |       |           |
| NAG61   | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>TPIp-EcaroL-pYX212t</i> ) + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(CCW12_BS123p-AtPAL2-FBA1t)</i> ) | NAG39 | This work |
| NAG62   | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>TPIp-EcaroL-pYX212t</i> ) + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(TDH3p-AtPAL2-FBA1t)</i> )        | NAG39 | This work |
| NAG63   | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>TPIp-EcaroL-pYX212t</i> ) + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(TDH3_BS23p-AtPAL2-FBA1t)</i> )   | NAG39 | This work |
| NAG64   | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>TPIp-EcaroL-pYX212t</i> ) + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(TEF1p-AtPAL2-FBA1t)</i> )        | NAG39 | This work |
| NAG65   | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>TPIp-EcaroL-pYX212t</i> ) + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) XI-2::( <i>TPIp-ACC1 S659A,S1157A -TDH2t</i> ) XII-3::( <i>(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t)</i> ) XI-3::( <i>(TEF1_BS123p-AtPAL2-FBA1t)</i> )  | NAG39 | This work |
| NAG39-2 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2::(GPM1p-AtPAL2-FBA1t)</i> + ( <i>(TDH3p-AtC4H-CYC1t)</i> + ( <i>tHXT7p-AtATR2-pYX212t</i> ) + ( <i>PGK1p-CYB5-ADH1t</i> ) X-3::( <i>(TPI1p-EcaroL-pYX212t)</i> + ( <i>ADH1t-ARO7 G141S-TEF1p</i> ) + ( <i>PGK1p-ARO4 K229L-CYC1t</i> ) X-4::( <i>(CYC1t-ARO1-TPI1p)</i> + ( <i>TDH3p-ARO2-ADH1t</i> ) + ( <i>TDH2t-ARO3-TEF1p</i> ) XII-4::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-1::( <i>(TDH3p-At4CL-ADH1t)</i> + ( <i>TDH2t-RsCHS-CCW12p</i> ) + ( <i>tHXT7p-PsCHI-FBA1t</i> ) XII-5::( <i>(pYX212t-PsCHI-PGKp)</i> + ( <i>TEF1p-RsCHS-FBA1t</i> ) + ( <i>CYC1t-At4CL-TPIp</i> ) XI-1::( <i>(pYX212t-PsCHI-PGKp)</i> +   | NAG39 | This work |

|       |   |       |           |
|-------|---|-------|-----------|
|       | $(TEF1p-RsCHS-FBA_t) XI-2:(TPIp-ACC1^{S659A,S1157A}-TDH2t) XII-3:(TDH2t-$<br>$matB-TDH3p) + (tHXT7p-matC-CYC1t) X-2:(TEF1-NLS_FapR-ADH1t)$  |       |           |
| NAG66 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(GPM1p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$        | NAG58 | This work |
| NAG67 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(CCW12p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$       | NAG59 | This work |
| NAG68 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(CCW12_BS2p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$   | NAG60 | This work |
| NAG69 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(CCW12_BS123p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$ | NAG61 | This work |
| NAG70 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(TDH3p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$        | NAG62 | This work |
| NAG71 | $MATa ura3-52 can1\Delta::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-$<br>$CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPIp-$<br>$EcaroL-pYX212t)+(ADH1t-ARO7^{G141S}-TEF1p)+(PGK1p-ARO4^{K229L}-CYC1t) X-$<br>$4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-$<br>$4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA_t)$<br>$XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-$<br>$FBA_t) XII-5::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) + (CYC1t-At4CL-$<br>$TPIp) XI-1::(pYX212t-PsCHI-PGKp)+(TEF1p-RsCHS-FBA_t) XI-2::(TPIp-ACC1$<br>$^{S659A,S1157A}-TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::$<br>$(TDH3_BS23p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)$   | NAG63 | This work |

|           |   |        |           |
|-----------|---|--------|-----------|
| NAG72     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 G141S-TEF1p)+(PGK1p-ARO4 K229L-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPIp) XI-1::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) XI-2::(TPIp-ACC1 S659A,S1157A -TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(TEF1p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)</i>   | NAG64  | This work |
| NAG73     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 G141S-TEF1p)+(PGK1p-ARO4 K229L-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPIp) XI-1::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) XI-2::(TPIp-ACC1 S659A,S1157A -TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(TEF1_BS123p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t)</i>   | NAG65  | This work |
| NAG74     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 G141S-TEF1p)+(PGK1p-ARO4 K229L-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPIp) XI-1::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) XI-2::(TPIp-ACC1 S659A,S1157A -TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(TDH3_BS23p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t) FDC1::( pYX212t-DCR1-TDH3p) + (tHXT7p-AGO1-CYC1t) XI-5::(TDH3_BS23p-FAS1_200bpshRNA-TDH2t)</i> | NAG71  | This work |
| NAG75     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 G141S-TEF1p)+(PGK1p-ARO4 K229L-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPIp) XI-1::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) XI-2::(TPIp-ACC1 S659A,S1157A -TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(TDH3_BS23p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t) FDC1::( pYX212t-DCR1-TDH3p) + (tHXT7p-AGO1-CYC1t) XI-5::(TDH3_BS23p-FAS1_200bpshRNA-TDH2t)</i> | NAG74  | This work |
| NAG76     | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-2:: TDH3p-AtC4H-CYC1t)+(tHXT7p-AtATR2-pYX212t)+(PGK1p-CYB5-ADH1t) X-3::(TPI1p-EcaroL-pYX212t)+(ADH1t-ARO7 G141S-TEF1p)+(PGK1p-ARO4 K229L-CYC1t) X-4::(CYC1t-ARO1-TPI1p)+(TDH3p-ARO2-ADH1t)+(TDH2t-ARO3-TEF1p) XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-1::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA) XII-5::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) + (CYC1t-At4CL-TPIp) XI-1::(pYX212t-PsCHI-PGKp)+( TEF1p-RsCHS-FBA) XI-2::(TPIp-ACC1 S659A,S1157A -TDH2t) XII-3::(TDH2t-matB-TDH3p) + (tHXT7p-matC-CYC1t) XI-3::(TDH3_BS23p-AtPAL2-FBA1t) X-2::(TEF1-NLS_FapR-ADH1t) FDC1::( pYX212t-DCR1-TDH3p) + (tHXT7p-AGO1-CYC1t) XI-5::(TDH3_BS23p-FAS1_250bpshRNA-TDH2t)</i> | NAG74  | This work |
| IMX581-N4 | <i>MATa ura3-52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3 XII-4::(TDH3p-At4CL-ADH1t) + (TDH2t-RsCHS-CCW12p)+(tHXT7p-PsCHI-FBA)</i>  | IMX581 | This work |

**Supplementary Table 2. Plasmids used in this study.**

| Plasmid ID | Relevant characteristics                                     | Origin    |
|------------|--|-----------|
| pCfB1018   | Template for AtPAL2 and AtC4H                                | (3)       |
| pCfB0848   | Template for AtATR2 and CYB5                                 | (3)       |
| pCfB0854   | Template for At4CL1  | (4)       |
| pCfB0826   | Template for ARO4 <sup>K229L</sup> and ARO7 <sup>G141S</sup> | (3)       |
| pCfB2747   | Template for EcoroL  | (3)       |
| pAD        | Template for ACC1 <sup>S659A, S1157A</sup>                   | (5)       |
| pFDA8      | Template for NLS-FapR  | (6)       |
| pX&Y19     | Template for pCCW12_BS2                                      | (7)       |
| pX&Y22     | Template for pCCW12_BS123                                    | (7)       |
| pX&Y31     | Template for pTDH3_BS23                                      | (7)       |
| pFDA7      | Template for pTEF1_BS123                                     | (6)       |
| pGK3       | Template for AGO1 and DCR1                                   | (8)       |
| pQC007     | 2μm ampR KIURA3 gRNA-XII-2.Y                                 | (2)       |
| pQC010     | 2μm ampR KIURA3 gRNA-XII-4.Y                                 | (2)       |
| pQC005     | 2μm ampR KIURA3 gRNA-X-3.Y                                   | (2)       |
| pQC008     | 2μm ampR KIURA3 gRNA-X-4.Y                                   | (2)       |
| pQC032     | 2μm ampR URA3 gRNA-XII-1.Y [2x]                              | (2)       |
| pQC033     | 2μm ampR URA3 gRNA-XII-5.Y [2x]                              | (2)       |
| pQC030     | 2μm ampR URA3 gRNA-XI-1.Y [2x]                               | (2)       |
| pQC130     | 2μm ampR URA3 gRNA-XI-2.Y [2x]                               | (2)       |
| pQC133     | 2μm ampR URA3 gRNA-XII-3.Y [2x]                              | (2)       |
| pQC029     | 2μm ampR URA3 gRNA-X-2.Y [2x]                                | (2)       |
| pQC006     | 2μm ampR KIURA3 gRNA-XI-3.Y                                  | (2)       |
| pQC009     | 2μm ampR KIURA3 gRNA-XI-5.Y                                  | (2)       |
| pMEL10     | 2μm ampR KIURA3 gRNA-CAN1.Y                                  | (2)       |
| pMC005     | 2μm ampR URA3 gRNA-AtPAL2.Y                                  | This work |

**Supplementary Table 3. Primers used in this study.**

| Plasmid ID  | Name                                   | Sequence (5'-3')   |
|---|--|--|
| <b>Primers for homologous regions for expression module integration</b> |  |  |
| P001  | XII-4 up-F                             | GTATCCGGCTGTTCTTCATAG                                      |
| P002  | XII-4 up-R (with TDH3p-F) <sup>a</sup> | CTTGAAATGGCAGTATTGATAATGATAAACCTCGATGCCATAGTATGTGTGATGG    |
| P003  | XII-4 dn-F (with FBA1t-R)              | CGAGTTCTTGTAAAGTCTTCATAGTAGCTTACTATCCCCATTAGAGTCAAATAAAG   |
| P004  | XII-4 dn-R                             | TTTCTGCCGTACCTGGATGGTCATTTC                                |
| P005  | XII-1 up-F                             | GTTGAGCTCTGCCCTCATGG                                       |
| P006  | XII-1 up-R1 (with TDH3p-F)             | CTTGAAATGGCAGTATTGATAATGATAAACCTCGAGAAAGAACCGAACCGATGCC    |
| P007  | XII-1 dn-F1 (with ADH1t-R)             | AAATCGCTCCCATTCAACCAATTGTAGATATGCCTTCCCGTAATCAACTGCAC      |
| P008  | XII-1 dn-R                             | CAATCCTCGCATTTCAGCTTC                                      |
| P009  | XII-1 up-R2 (with pYX212t-R)           | GCTCCCTTAGGGTCCGATTAGTGGTTACGGCGAAAGAACCGAACCGATGC         |
| P010  | XII-1 dn-F2 (with FBA1t-R)             | CGAGTTCTTGTAAAGTCTTCATAGTAGCTTACTGTTAGTGCCTGTCTGAG         |
| P011  | XII-5 up-F                             | GTAGTGATCATTGGCTTAAC                                       |
| P012  | XII-5 up-R1 (with pYX212t-R)           | GCTCCCTTAGGGTCCGATTAGTGGTTACGGCGTGACAATAAATTCAAACCGGT      |
| P013  | XII-5 dn-F1 (with TPI1p-F)             | ATTCTAAGTAAGTAAATATCCGTAATCTTAAACCAACTCAGAAGTTGACAGC       |
| P014  | XII-5 dn-R                             | CTCTTTGCCTTCAAAAAAAG                                       |
| P015  | XII-5 up-R2 (with CYC1t-R)             | GGACGCTCGAAGGCTTAATTGCGGCCGGTACCCGTGACAATAAATTCAAACCGGT    |
| P016  | XII-5 dn-F2 (with FBA1t-R)             | CGAGTTCTTGTAAAGTCTTCATAGTAGCTTACTCAACTCAGAAGTTGACAGC       |
| P017  | XII-5 dn-F3 (with PGK1p-F)             | ATGCCTATTGTGCAGATGTTATAATATCTGTGCGTCAACTCAGAAGTTGACAGC     |
| P018  | XII-5 up-R3 (with TEF1p-F)             | AGAGTAAAAAAGGAGTAGAACATTGAAAGCTATGTGACAATAAATTCAAACCGGT    |
| P019  | XI-1 up-F                              | ATTGTGTGAAGGAATAGTGACG                                     |
| P020  | XI-1 up-R1 (with pYX212t-R)            | GCTCCCTTAGGGTCCGATTAGTGGTTACGGCCAATGGGCTTGGTATTCCG         |
| P021  | XI-1 dn-F1 (with TPI1p-F)              | ATTCTAAGTAAGTAAATATCCGTAATCTTAAACTTCTGGCATTGGCAAATC        |
| P022  | XI-1 dn-R                              | AAGAGCCGAGTCCCCATCAG                                       |
| P023  | XI-1 up-R2 (with CYC1t-R)              | GGACGCTCGAAGGCTTAATTGCGGCCGGTACCCCAATGGGCTTGGTATTCCG       |
| P024  | XI-1 dn-F2 (with FBA1t-R)              | CGAGTTCTTGTAAAGTCTTCATAGTAGCTTACTTTCTGGCATTGGCAAATC        |
| P025  | XI-1 dn-F3 (with PGK1p-F)              | ATGCCTATTGTGCAGATGTTATAATATCTGTGCGTCTTCTGGCATTGGCAAATC     |
| P026  | XI-1 up-R3 (with TEF1p-F)              | AGAGTAAAAAAGGAGTAGAACATTGAAAGCTATCAATGGGCTTGGTATTCCG       |
| P027  | XII-2 up-F                             | CGGCATGCAAACATCTACAC                                       |
| P028  | XII-2 up-R (with GPM1p-F)              | GCTCACAAATCTTAAAGTCATACATTGACAGACTACATAACCGCTTACACGGAAAG   |
| P029  | XII-2 dn-F (with PGK1p-F)              | GCAAATGCCTATTGTGCAGATGTTATAATATCTGTGCGTCTACTATGGCGACTCTCTC |
| P030  | XII-2 dn-R                             | GAGCGAACGTAAGAGAGGTTAATG                                   |
| P031  | X-3 up-F                               | CGAGATCTTGTGTTCGGTTACC                                     |
| P032  | X-3 up-R (with TPI1p-F)                | CTAAGTAAGTAAATATCCGTAATCTTAAACGTCCTCGTATGTCGGCTCTCGC       |
| P033  | X-3 dn-F (with CYC1t-R)                | GACGCTCGAAGGCTTAATTGCGGCCGGTACCCCTGTGTCGCCGTTTCTAAAGGC     |
| P034  | X-3 dn-R                               | GAGGTGGTTATTGATCACCGGA                                     |
| P035  | X-4 up-F                               | CCCAAAGCTAACAGACTCCCATT                                    |
| P036  | X-4 up-R (with CYC1t-R)                | GACGCTCGAAGGCTTAATTGCGGCCGGTACCCCTGCTCTGAATGGCGACAG        |
| P037  | X-4 dn-F (with TEF1p-F)                | GAAGAGTAAAAAAGGAGTAGAACATTGAAAGCTATAACAGGCATGGGAAGATTG     |
| P038  | X-4 dn-R                               | CTGGTGAGGATTACGGTATGA                                      |
| P039  | X-2 up-F                               | CGTCTATGAGGAGACTGTTAGTTG                                   |
| P040  | X-2 up-R (with GPM1p-F)                | GCTCACAAATCTTAAAGTCATACATTGACAGACTAGACCACTCGAGAGCAAGTTG    |
| P041  | X-2 dn-F (with CYC1t-R)                | GACGCTCGAAGGCTTAATTGCGGCCGGTACCCCTGCATAATGGCCTCAC          |
| P042  | X-2 dn-R                               | CTCGCCAAGGCATTACCATC                                       |
| P043  | XI-2 up-F                              | TAACTCTTCGTATGAGGATTTTC                                    |
| P044  | XI-2 up-R                              | TTCTATGGCACATTCTGTGTTG                                     |
| P127  | XI-2 dn-F                              | CCACAAGTAAAGCTCGTTGAC                                      |
| P128  | XI-2 dn-R                              | ATGGTTGAAAAGGTTACAGAGG                                     |
| P129  | XII-3 up-F                             | TGTGCCCTTAAATTATCATATAC                                    |
| P130  | XII-3 up-R (with TDH2t-R)              | TAAAGCACTTAGTATCACACTAATTGGCTTTCGCGAATGAGCAGGTACCCCTTA     |
| P131  | XII-3 dn-F (with CYC1t-R)              | GGACGCTCGAAGGCTTAATTGCGGCCGGTACCCGCATAGAGCTAATTAGTTGAG     |

|      |                                   |   |
|------|-----------------------------------|---|
| P132 | <i>XII-3 dn-R</i>                 | GAACTTACAAGCTGATTTGGT                                   |
| P133 | <i>X-2 up-R (with TEF1p-F)</i>    | AGAGTAAAAAAGGAGTAGAACATTGAAAGCTATGACCACCTCGAGAGCAAGTTG  |
| P134 | <i>X-2 dn-F (with ADH1t-R)</i>    | AAATCGCTCCCCATTCACCCAATTGTAGATATGCCCTGCATAATCGGCCTCAC   |
| P135 | <i>XI-3 up-F</i>                  | AGTTACTTGCTCATGCGTTGC                                   |
| P136 | <i>XI-3 up-R1 (with GPM1p-F)</i>  | GCTCACAAATCTTAAAGTCATACATTGACAGACTAAATCAGACGCACGCTTGGC  |
| P137 | <i>XI-3 dn-F (with FBA1t-R)</i>   | CGAGTTCTTGAAAGTCTTCATAGTAGCTTACGTGGATTGAGCCAGCA         |
| P138 | <i>XI-3 dn-R</i>                  | TGAGAACTCGGACCGACAGT                                    |
| P139 | <i>XI-3 up-R2 (with CCW12p-F)</i> | GCCCCCTTGGACTAACCGTGTGGTCATGGGTGGAATCAGACGCACGCTTGGC    |
| P140 | <i>XI-3 up-R3 (with TDH3p-F)</i>  | CTTGAATGGCAGTATTGATAATGATAAACTCGAAATCAGACGCACGCTTGGC    |
| P141 | <i>XI-3 up-R4 (with TEF1p-F)</i>  | AGAGTAAAAAAGGAGTAGAACATTGAAAGCTATAATCAGACGCACGCTTGGC    |
| P142 | <i>FDC1 up-F</i>                  | CTGAGCATTATTACGTTACTCAAC                                |
| P143 | <i>FDC1 up-R (with pYX212t-R)</i> | CCCTTAGGGTCCGATTAGTGGTTACGGCTTGAATATATAAATTGACAATTCTTTG |
| P144 | <i>FDC1 dn-F (with CYC1t-R)</i>   | GGACGCTCGAAGGCTTAATTGCGGCCGTACCCCTGCCATAGACTTCTACGG     |
| P145 | <i>FDC1 dn-R</i>                  | GTCCATTATTTTATGTGCTGTC                                  |
| P146 | <i>XI-5 up-F</i>                  | GCGGAGAAGTCGTTGATAGC                                    |
| P147 | <i>XI-5 up-R1 (with TDH3p-F)</i>  | CTTGAATGGCAGTATTGATAATGATAAACTCGATGGTCACGGAGTTATGG      |
| P148 | <i>XI-5 dn-F (with TDH2t-R)</i>   | TAAGCACTTAGTATCACACTAATTGGCTTCGCTTGTAAAACAGGTATTGGCTGC  |
| P149 | <i>XI-5 dn-R</i>                  | GATCATAGATCCGGCACTTAGAG                                 |

#### Primers for amplification of promoters and terminators

|      |                                  |   |
|------|----------------------------------|---|
| P045 | <i>TDH3p-F</i>                   | TCGAGTTATCATTATCAATACTGCC                                 |
| P046 | <i>TDH3p-R</i>                   | CATTGTTGTTATGTGTGTTATTGCA                                 |
| P047 | <i>ADH1t-F</i>                   | GCGAATTCTTATGATTATGATT                                    |
| P048 | <i>ADH1t-R</i>                   | GCATATCTACAATTGGGTGAAATGG                                 |
| P049 | <i>TDH2t-F1</i>                  | ATTTAACCTTAAGTTACTTAATGATTAG                              |
| P050 | <i>TDH2t-R1 (with ADH1t-R)</i>   | AAATCGCTCCCCATTCACCCAATTGTAGATATGCGCGAAAGCCAATTAGTGTGATAC |
| P051 | <i>CCW12p-F</i>                  | CCACCCATGAACCACACGG                                       |
| P052 | <i>CCW12p-R</i>                  | CATTGTTATTGATATAGTGTAAAGCGAATG                            |
| P053 | <i>tHXT7p-F1 (with CCW12p-F)</i> | GCCCCCTTGGACTAACCGTGTGGTCATGGGTGGCTCGTAGGAACAATTGCGG      |
| P054 | <i>tHXT7p-R</i>                  | CATTTTTGATAAAAATTAAAAAAACTTTTG                            |
| P055 | <i>FBA1t-F</i>                   | GTAAATTCAAATTGATATAGTTTT                                  |
| P056 | <i>FBA1t-R</i>                   | GATACCGTCGACCTCGAGTC                                      |
| P057 | <i>GPM1p-F</i>                   | TAGTCGTGCAATGTATGACTTAAGA                                 |
| P058 | <i>GPM1p-R</i>                   | CATTGTTTATTGTAATATGTTGTTGTT                               |
| P059 | <i>CYC1t-F</i>                   | GATACCGTCGACCTCGAGTC                                      |
| P060 | <i>CYC1t-R1</i>                  | GGGTACCGGCCGCAAATTAA                                      |
| P061 | <i>pYX212t-F</i>                 | TAGGGCCCACAAGCTTACG                                       |
| P062 | <i>pYX212t-R1 (with ADH1t-R)</i> | CAAATCGCTCCCCATTACACCAATTGTAGATATGCCGTAAACCACTAAATCGGA    |
| P063 | <i>ADH1t-F</i>                   | GCGAATTCTTATGATTATGATT                                    |
| P064 | <i>ADH1t-R</i>                   | GCATATCTACAATTGGGTGAAATGG                                 |
| P065 | <i>PGK1p-F</i>                   | ACGCACAGATATTATAACATCTGC                                  |
| P066 | <i>PGK1p-R</i>                   | CATTTGTTATTGTTGAAAAAGTAGATAA                              |
| P067 | <i>TPI1p-F</i>                   | GTTAAAGATTACGGATATTAACTAC                                 |
| P068 | <i>TPI1p-R</i>                   | CATTTTAGTTATGTATGTTTTGTAG                                 |
| P069 | <i>TEF1p-F1</i>                  | ATAGCTCAAATGTTCTACTCCT                                    |
| P070 | <i>TEF1p-R</i>                   | CATTTGTAATTTAGATTAGATTGC                                  |
| P071 | <i>TEF1p-F2 (with PGK1p-F)</i>   | ATGCCTATTGTCAGATGTTATAATCTGTGCGTATAGCTTCAAAATGTTCTACTCCT  |
| P072 | <i>PGK1p-F</i>                   | ACGCACAGATATTATAACATCTGC                                  |
| P073 | <i>PGK1p-R</i>                   | CATTTGTTATTGTTGAAAAAGTAGATAA                              |
| P074 | <i>pYX212t-R2</i>                | GCCGTAACCAACTAAATCGGA                                     |
| P075 | <i>TDH2t-R2</i>                  | GCGAAAGCCAATTAGTGTGATAC                                   |
| P076 | <i>tHXT7p-F2</i>                 | CTCGTAGGAACAATTGCGG                                       |
| P077 | <i>tHXT7p-F3 (with TDH3p-F)</i>  | CTTGAATGGCAGTATTGATAATGATAAACTCGACTCGTAGGAACAATTGCGG      |
| P078 | <i>CYC1t-R2 (with FBA1t-R)</i>   | TCGAGTTCTTGAAAGTCTTACGTAGCTACGGTACCGGGCAAAATTAA           |

|      |   |  |
|------|---|--|
| P079 | <i>TEF1p-F3</i> (with <i>XI-2_up</i> )  | CGGGGTGTAACTCAACAGAAAAATGTGCCATAGAAATAGCTTCAAATGTTCTACTCCT |
| P080 | <i>TEF1p-R</i> (with <i>mACC1_up</i> )  | GAGAAGACTCGAATAAGCTTCTCGCTATTGTAACTTAAACCTAGATTAGATTGC     |
| P081 | <i>TDH2t-F2</i> (with <i>mACC1_dn</i> ) | AAGAAAATTGTTGAAGACTTGAATAAAATTAACTCTTAAGTTACTTTAATGATTAG   |
| P082 | <i>TDH2t-R3</i> (with <i>XI-2_dn</i> )  | CAACTGATCAACTGGTCAACGAGCTTACTTGTGGCGAAAGCCAATTAGTGTGATAC   |

**Primers for amplification of genes**

|      |  |  |
|------|--|--|
| P164 | <i>At4CL1-F</i> (with <i>TDH3p-R</i> )   | ACTTAGTTCGAATAAACACACATAAAACAAACAAAATGGCTCCACAAGAACAG          |
| P165 | <i>At4CL1-R</i> (with <i>ADH1t-F</i> )   | TTAATAATAAAATCATAAATCATAAGAAATCGCTCACAAACCGTTAGCCAAC           |
| P166 | <i>RsCHS-F</i> (with <i>CCW12p-R</i> )   | CTGTCATTGCTTAAACACTATATCAATAAAACAAATGGTTACTGTTGAAGATGTTAG      |
| P167 | <i>RsCHS-R</i> (with <i>TDH2t-F</i> )    | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATTAAAGTACACAATGAATGAAAC      |
| P168 | <i>PsCHI-F</i> (with <i>tHXT7p-R</i> )   | CAAAACAAAAAGTTTTTAATTAACTCAAAAATGGCAAACACCACATCTG              |
| P169 | <i>PsCHI-R</i> (with <i>FBA1t-F</i> )    | TCATTAACAAACTATATCAATTAAATTGAATTAACTTAACTTAAACAATTGAGAAATTCTGC |
| P170 | <i>HaCHS-F</i> (with <i>CCW12p-R</i> )   | CTGTCATTGCTTAAACACTATATCAATAAAACAAATGGTTACTGTTGAAGAAG          |
| P171 | <i>HaCHS-R</i> (with <i>TDH2t-F</i> )    | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATTAAATTAAAGCAACTGAATG        |
| P172 | <i>SmCHS-F</i> (with <i>CCW12p-R</i> )   | CTGTCATTGCTTAAACACTATATCAATAAAACAAATGGTTACTGTTGAAGAATAC        |
| P173 | <i>SmCHS-R</i> (with <i>TDH2t-F</i> )    | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATTAAAGCTGCAACAGAACATGC       |
| P174 | <i>PhCHI-F</i> (with <i>tHXT7p-R</i> )   | CAAAACAAAAAGTTTTTAATTAACTCAAAAATGTCTCCACCAGTTAG                |
| P175 | <i>PsCHI-R</i> (with <i>FBA1t-F</i> )    | TCATTAACAAACTATATCAATTAAATTGAATTAACTTAAACACCAATAACTGGG         |
| P176 | <i>SmCHI-F</i> (with <i>tHXT7p-R</i> )   | CAAAACAAAAAGTTTTTAATTAACTCAAAAATGGCTGCTGTACTAAATTG             |
| P177 | <i>SmCHI-R</i> (with <i>FBA1t-F</i> )    | TCATTAACAAACTATATCAATTAAATTGAATTAACTGGCTGCTGTACTAAATTG         |
| P178 | <i>AtPAL2-F</i> (with <i>GPM1p-R</i> )   | CCAAACAAACACACATATTACAATAAAACAAATGGATCAAATCGAAGCTATGTT         |
| P179 | <i>AtPAL2-R</i> (with <i>FBA1t-F</i> )   | CTCATTAAAAACTATATCAATTAAATTGAATTAACTCAGCAGATAGGAATAGGAGCACC    |
| P180 | <i>AtC4H-F</i> (with <i>TDH3p-R</i> )    | GTTCGAATAAACACACATAAAACAAACAAATGGACTTGTGTTGGAAAAG              |
| P181 | <i>AtC4H-R</i> (with <i>CYC1t-F</i> )    | CATAACTATTACATGACTCGAGGTGACGGTATCTAACAGTTCTGGCTCATAC           |
| P182 | <i>AtATR2-F</i> (with <i>tHXT7p-R</i> )  | CAAAACAAAAAGTTTTTAATTAACTCAAAAATGTCTCCTCTTCTCATCATC            |
| P183 | <i>AtATR2-R</i> (with <i>pYX212t-F</i> ) | CCGGGTGACCGTAAGCTTGTGGCCCTATCACCAGACATCTCTCAAGTATCTAC          |
| P184 | <i>CYB5-F</i> (with <i>PGK1p-R</i> )     | GTAATTATCTACTTTACAACAAATATAACAAATGCTAAAGTTACAGTACCAAG          |
| P185 | <i>CYB5-R</i> (with <i>ADH1t-F</i> )     | TTAATAATAAAATCATAAATCATAAGAAATTGCTCATTGTTCAACAAATAAAAGC        |
| P186 | <i>EcoroL-F</i> (with <i>TPI1p-R</i> )   | CTATAACTACAAAAACACATACATAAAACTAAACATGACACAACCTCTTCTGATC        |
| P187 | <i>EcoroL-R</i> (with <i>pYX212t-F</i> ) | GATACCCGGTGCACGCGTAAGCTTGTGGCCCTATCAACAAATTGATCGTCTGTGCC       |
| P188 | <i>ARO7*-F</i> (with <i>TEF1p-R</i> )    | CATAGCAATTCTAACTCAAGTTTAATTACAAAATGGATTACAAAACCAGA             |
| P189 | <i>ARO7*-R</i> (with <i>ADH1t-F</i> )    | TATTAAATAATAAAATCATAAATCATAAGAAATTGCTTACTCTTCAACCTCTT          |
| P190 | <i>ARO4*-F</i> (with <i>PGK1p-R</i> )    | GTAATTATCTACTTTACAACAAATATAACAAATGAGTGAATCTCAATTGTTG           |
| P191 | <i>ARO4*-R</i> (with <i>CYC1t-F</i> )    | CATAACTATTACATGACTCGAGGTGACGGTATCTTCTGTAACTCTCTT               |
| P192 | <i>ARO1-F</i> (with <i>TPI1p-R</i> )     | CTATAACTACAAAAACACATACATAAAACTAAACATGTCAGTTAGCCAAAGTC          |
| P193 | <i>ARO1-R</i> (with <i>CYC1t-F</i> )     | CATAACTATTACATGACTCGAGGTGACGGTATCTTCTGTAAACGGCATCA             |
| P194 | <i>ARO2-F</i> (with <i>TDH3p-R</i> )     | GTTTCGAATAAACACACATAAAACAAACAAATGTCACGTTGGAAACTG               |
| P195 | <i>ARO2-R</i> (with <i>ADH1t-F</i> )     | CTTATTTAATAATAAAATCATAAATCATAAGAAATTGCTTAATGAACCACGGATCTGA     |
| P196 | <i>ARO3-F</i> (with <i>TEF1p-R</i> )     | CATAGCAATTCTAACTCAAGTTTAATTACAAAATGTTCTTAAACAGATCACGC          |
| P197 | <i>ARO3-R</i> (with <i>TDH2t-F</i> )     | CTAAATCATTAAAGTAACCTAAGGAGTTAACTTAACTGTTCAAGGCCCTTC            |
| P198 | <i>PHA2-F</i> (with <i>GPM1p-R</i> )     | CCAAACAAACACACATATTACAATAAAACAAATGGCCAGCAAGACTTGAGG            |
| P199 | <i>PHA2-R</i> (with <i>CYC1t-F</i> )     | ACTAATTACATGACTCGAGGTGACGGTATCTTATTGTGATAATCTCTCATTCTG         |
| P200 | <i>At4CL1-F</i> (with <i>TPI1p-R</i> )   | TCTATAACTACAAAAACACATACATAAAACTAAACATGCTCCACAAGAACAG           |
| P201 | <i>At4CL1-R</i> (with <i>CYC1t-F</i> )   | CATAACTATTACATGACTCGAGGTGACGGTATCTCACAAACCGTTAGCCAAC           |
| P202 | <i>RsCHS-F</i> (with <i>TEF1p-R</i> )    | AAGCATAGCAATTCTAAAGTTTAATTACAAAATGGTTACTGTTGAAGATGTTAG         |
| P203 | <i>RsCHS-R</i> (with <i>FBA1t-F</i> )    | TCATTAACAAACTATATCAATTAAATTGAATTAACTTAAAGTACACAATGAATGAAAC     |
| P204 | <i>PsCHI-F</i> (with <i>PGK1p-R</i> )    | AGTAATTATCTACTTTACAACAAATATAACAAATGGCAAACACCACATCTG            |
| P205 | <i>PsCHI-R</i> (with <i>pYX212t-F</i> )  | ACCCGGGTGACCGTAAGCTTGTGGCCCTATTATTTAACAAATTGAGAAATTCTTG        |
| P206 | <i>mACC1_up 500bp-F</i>                  | ATGAGCGAAGAAAGCTTATTG  |
| P207 | <i>mACC1_up 500bp-R</i>                  | CAATAGTGGATTCTCGGAGGC  |
| P208 | <i>mACC1_dn 500bp-F</i>                  | ATCGTAGAGAGAGAACTATTGCC  |
| P209 | <i>mACC1_dn 500bp-R</i>                  | TTATTCAGAAAGTCTCAACAAATTCTTATC                                 |
| P210 | <i>matB-F</i> (with <i>TDH3p-R</i> )     | CTTAGTTCGAATAAACACACATAAAACAAACAAATGTCATACTTTGTTGATGCAATG      |
| P211 | <i>matB-R</i> (with <i>TDH2t-F</i> )     | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATTAAAGTCTTGTGACAAATCAGC      |

|      |   |  |
|------|---|--|
| P212 | <i>matC-F (with tHXT7p-R)</i>                       | CAAAAACAAAAAGTTTTTAATTTAATCAAAAATGGTATCGAATTGTTCTATC       |
| P213 | <i>matC-R (with CYC1t-F)</i>                        | TAACATAATTACATGACTCGAGGTCGACGGTATCTAAACTAACCTGGAACAACAAAAC |
| P223 | <i>AtPAL2-F (with CCW12p-R)</i>                     | CTGTCATTGCTTAAACACTATATCAATAACAAAATGGATCAAATCGAAGCTATG     |
| P224 | <i>AtPAL2-F (with TEF1p-R)</i>                      | TAGCAATCTAATCTAAGTTTAATTACAAAATGGATCAAATCGAAGCTATG         |
| P225 | <i>AtPAL2-F (with TDH3p-R)</i>                      | TTTCGAATAAACACACATAAAACAAACAAAATGGATCAAATCGAAGCTATG        |
| P226 | <i>NLS_FapR-F (with TEF1p-R)</i>                    | GCAATCTAATCTAAGTTTAATTACAAAATGCCAAGAAGAAGAGAAAGGTTAG       |
| P227 | <i>NLS_FapR-R (with ADH1t-F)</i>                    | TTAATAATAAAATCATAAATCATAGAAATTGCTTATGAATGTTGAACGATACATG    |
| P228 | <i>DCR1-F (with TDH3p-R)</i>                        | GATACCCGGGTGACCGTAAGCTGTGGGCCCTATCACAGATTGTTGAATGCC        |
| P229 | <i>DCR1-R (with pYX212t-F)</i>                      | ACTTAGTTCGAATAAACACACATAAAACAAACAAAATGAATAGAGAAAAAGCGCC    |
| P230 | <i>AGO1-F (with tHXT7p-R)</i>                       | CAAAAACAAAAAGTTTTTAATTTAATCAAAAATGTCATCCAATTGGAGGAG        |
| P231 | <i>AGO1-R (with CYC1t-F)</i>                        | CATAACTAATTACATGACTCGAGGTCGACGGTATCTCATATGTAGTACATGATGTCAG |
| P232 | <i>FAS1_sense 200bp-F (with TDH3p-R)</i>            | TTTCGAATAAACACACATAAAACAAACAAAATGGACGCTTACTCCACAAG         |
| P233 | <i>FAS1_sense 200bp-R (with rad9_intron1-F)</i>     | GAAAAAAAGTTCCAACACACACCTGCTGCAGCAAACCCTTCAG                |
| P234 | <i>rad9_intron1-F</i>                               | CAGGTGTGTTGGAACTTTTCAAACCTTACTAAACATTGAAACTATTGGTAAAGATA   |
| P235 | <i>rad9_intron1-R</i>                               | TATCTTACCAATTAGTTCAATGTTAGTAAGGTTGAAAAAAGTCCAACACACCTG     |
| P236 | <i>FAS1_antisense 200bp-F (with rad9_intron1-R)</i> | AACATTGAAACTAATTGGTAAAGATACTGCAGCAAACCCTTCAG               |
| P237 | <i>FAS1_antisense 200bp-R (with TDH2t-F)</i>        | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATATGGACGCTTACTCCACAAG    |
| P238 | <i>FAS1_sense 250bp-F (with TDH3p-R)</i>            | TTTCGAATAAACACACACATAAAACAAACAAAATGGACGCTTACTCCACAAG       |
| P239 | <i>FAS1_sense 250bp-R (with rad9_intron1-F)</i>     | GAAAAAAAGTTCCAACACACACCTGAGACCTGATCGAATTGACC               |
| P240 | <i>FAS1_antisense 250bp-F (with rad9_intron1-R)</i> | AACATTGAAACTAATTGGTAAAGATAAGACCTGATCGAATTGACC              |
| P241 | <i>FAS1_antisense 250bp-R (with TDH2t-F)</i>        | AAACTAAATCATTAAAGTAACCTAAGGAGTTAAATATGGACGCTTACTCCACAAG    |

#### Primers for *AtPAL2* gene deletion and repair oligos

|      |                            |  |
|------|----------------------------|--|
| P214 | <i>Vector backbone-F</i>   | GATCATTATCTTCACTGCGGAGAAG  |
| P215 | <i>Vector backbone-R</i>   | GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTC   |
| P216 | <i>Sequencing-F</i>        | CACCTTCGAGAGGGACGATG   |
| P217 | <i>Sequencing-R</i>        | GCTGGCCTTTGCTCACATG  |
| P218 | <i>AtPAL2_gRNA</i>         | TGCCCATGTTCGCGTTCGAAACTTCTCCGAGTGAAGATAATGATCAATGGTACA<br>GCTGTTGGTCGTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGTCCGTTATCAA<br>C  |
| P219 | <i>Repair Fragment 1-F</i> | GCCATTTTTCTGTATCGGGCCCTCCTACTGCTCTCCCGTGTACCGCGTTATG<br>AATTTTATTCATTCTGGAACTCTCGAGTTCTGAAAGTCTTCATAGTAGCTTAC<br>GTAAGCTACTATGAAAGACTTACAAGAACCTCGAAGAGTCCAGAATGAAATAAAATT |
| P220 | <i>Repair Fragment 1-R</i> | CATAACCGCGTTACACGGAAGGGAGAGCAGTAAGGAGGGCCGATACAGAAAAAAATG<br>GC  |
| P221 | <i>Repair Fragment 2-F</i> | GCCATTTTTCTGTATCGGGCCCTCCTACTGCTCTCCCGTGTACCGCGTTATG<br>CTACTATCGGCAGCTCTCGAAATTCTTAACCGCGCTTGACTGCGCTAACGCT<br>AGCGTTAGACCGCAGTACAAGGACGCGTTAAGAAAAATTGAGAGAGTCGCCGATAGT  |
| P222 | <i>Repair Fragment 2-R</i> | AGCATAACCGCGTTACACGGAAGGGAGAGCAGTAAGGAGGGCCGATACAGAAAAAA<br>TGGC   |

<sup>a</sup> short overlap to the fragments in the parentheses were included in corresponding primers.

**Supplementary Table 4. Codon optimized genes used in this study.**

| Gene         | Sequence (5'-3')  |
|--------------|---|
| <i>HaCHS</i> | ATGGTTACTGTTGAAGAAGTTAGAAAAGCACAAAGAGCTGAAGGTCCAGCAACTGTTATGGCTATTGGTACAGCAGTT<br>CCACCAAATTGTTGATCAAGCTACTTACCCAGATTACTACTTCAGAATTACAATTCTGAACATAAGGCAGAATTAA<br>AGGAAAAGTCCAAAGAACATGTGTGATAAATCACAAATTAAAGAAAAGATATGTACTTGAATGAAGAAGTTAAAAGA<br>AAATCCAATATGTGTGATCATGGCCATCTTAGATGCTAGACAAGATATCGTTGAAGTCCAAATTGTT<br>GGTAAAGAAGCTGCTTAAAGCAATTAAAGAATGGGTCAACCAAGTCTAAGATCACTCATTGGTTCTGACT<br>ACATCAGGTGTTGATATGCCAGGTGCTATTACCAATTGACAAAATTGTTGGTTAAAGACCATCAGTTAAGAGATTG<br>ATGATGTAACCAACAAGGTTGTTGAGGTGGTACAGTTGAGATTGGCTAAAGATTGGCAGAAAACAATAAGGGT<br>GCTAGAGTTTGGTTGTTCTGAAATCACTGCTTACTTTAGAGGTCACACTGATAACACATTGGATTCTAG<br>TTGGTCAAGCATTGTTGGTACGGTGCTGCTGCTATTATTGGTCTGATCCAATTCCAGAAGTGGTAAAGGCCATT<br>GTTCGAATTGGTTCTGCTGCTCAAACATTACCAATTGAGGTCATTGACAGGTTGCTATTGATGGTCAATTGAGAGATTG<br>TTAACATTCCATTGTTGAAGGATGTTCCAGGTTGATCTCTAAAATGTTGAAAATCATTGACTGAAGCCTTAAAC<br>CATTGGGTACTCTGATTGAACTCTTATTGGATTGCTCATCCAGGTGGTCCAGCAATTGGATCAAGTGAAG<br>CTAAATTGCTTAAAGCCAGAAAATTGAGAGCAACAGACATGTTGCTAGAAATCGTAACATGCTCAGCTT<br>TGTTTGTATTAGATGAAATGAGAAAAGCTAAGGAAGATGGTTGAAGACTACAGGTGAAGGTATTGAATG<br>GGGTGTTTGTGTTGGTTGGTCCAGGTTACTGTTGAAACAGTTGTTGATTGCTATTGCTATTAAATTAA |
| <i>RsCHS</i> | ATGGTTACTGTTGAAGATGTTAGAAGAGCACAAAGAGCTGAAGGTCCAGCAACAGTTATGGCTATTGGTACTGCAAC<br>ACCATCTAATTGTTGATCAACTTACCCAGATTCTACTTAACTGTTGAAATCAAGGAAATTCTGAACATAAGGCTGAATTAA<br>AGGAAAAGTCCAAAGAACATGTGTGATAAATCAATGTTAAAGAAAAGATATGTACTTAACAGGAAATTGG<br>AAACCCATCTGTTGTGATATATGGCCATCATTAGATGCAAGACAAGATATGGTTGTTGAAGTCCAAATTG<br>GGTAAAGAAGCTGCAACTAAAGCTTAAAGAATGGGTCAACCAAGTCTAAGATCACACATTGGTTCTGACT<br>ACATCAGGTGTTGATATGCCAGGTGCTGAGATTACCAATTGACTAAATTGTTGGTTAAAGACCATCTGTTAAGAGATTG<br>ATGATGTAACCAACAAGGTTGTTGCTGGTGTACAGTTGAGATTGGCTAAAGATTGGCAGAAAACAATAAGGGT<br>GCTAGAGTTTGGTTGTTCTGAAATCACTGCTTACTTTAGAGGTCATCTGATAACACATTGGATTCTGG<br>TTGGTCAAGCATTGTTGGTACGGTGCTGCTGCTATTATTGGTGTGCTGAGATCCAGTCCAGAAGTGGTAAAGGCCATT<br>TGTTCGAATTGGTTCTGCTGCTCAAACATTACCAATTGAGGTCATTGCTGATTGATGGTCAATTGAGAGATTG<br>TTTGACATTCCATTGTTGAAGGATGTTCCAGGTTGATCTCTAAAATGTTGAAAAGCATTGACTGAAGCATTCAA<br>CCATTGGGTATTCTGATTGAACTCTTATTGGATTGCTCATCCAGGTGGTCCAGCAATTGGATCAAGTGAAT<br>TGAAATTATCTTGAAGCCAGAAAATTGAGAGCTACAGACATGTTGCTAGAAATCGTAACATGCTCAGCATG<br>TGTTTGTATTGGATGAAATGAGAAAATTGCTGTAAGAAGGTTAAAACACAGGTGAAGGTTAGAATGG<br>GGTGTGTTGTTGGTTGGTCCAGGTTACTGTTGAAACAGTTGTTGATTGCTATTGCTATTAA       |
| <i>SmCHS</i> | ATGGTTACTGTTGAAGAACATACAGAAAGGCACAAAGAGCTGAAGGTCCAGCAACAGTTATGGCTATTGGTACTCTACA<br>CCATCAAATTGTTGATCAATCTGCTTACCCAGATTACTTAACTGTTGAAATCAAGAACATAAGACAGAAATTAA<br>GGAAAAGTTAAAGAACATGTGAAAATCTATGTTAAAGAAAAGATATGTACTTAACAGGAAATTGG<br>AAATCTAATATGTGTGATATATGGCTCATCATTAGATGCAAGACAAGATATCGTTGTTGAAGTCCAAATTG<br>GTAAAGAAGCTGCACAAAAGCTTAAAGAATGGGTCAACCAAGTCTAAGATCACTCATTTGGTTCTGACTA<br>CATCAGGTGTTGATATGCCAGGTGCTGATTACCAATTGACAAAATTGTTGGTTAAAGACCATCTGTTAAGAGATTG<br>GATGTAACCAACAAGGTTGTTGCTGGGGTACAGTTGAGATTGGCAAAGATTGGCTGAAAACAATAAGGGTGC<br>AAGAGTTTGGTTGTTCTGAAATCACTGCTTACTTTAGAGGTCATCTGATAACATTTGGATTCTGGT<br>GGTCAAGCATTGTTGGTACGGTGCTGCTGTTATTGGTCTGATCCAATTTCAGGTGTTGAAAGACCATTA<br>TTTGAATTGGTTCTGCTGCTCAAACATTGTTACCAATTGAGGTCATTGATGGTCAATTGAGAGAAGTGGT<br>TGACATTCCATTGTTGAAGGATGTTCCAGGTATCATCTCTAAAATGATAACATTGAGGTCATTGAGGTCATTCAA<br>TTGGGTACTCTGATTGAACTCTTATTGGATTGACATCCAGGTGGTCCAGCTATTGGATCAAGTGAATTG<br>AATTGGGTTGAACCCAGAAAATTGAGAGCAACTAGAGAAGTTGCTAACTACGGTAACATGCTCAGCTG<br>TTTGTGTTATTGGATGAAATGAGAAAAGCATCAGCTAAACAGGTTACCAACTACAGGTGAAGGTTACAATGG<br>GTGTTTGTGTTGGTTGGTCCAGGTTACTGTTGAAACATTAGTTGCTATTGCTATTGCTGAGCTTAA                            |
| <i>PhCHI</i> | ATGTCTCCACCAGTTCACTAACAGATGCAAGTGAAGAAACTATGCTTTCAGCAACAGTTAACCCAGCAGTTCTA<br>CTAATACATTGTTTAGCTGGTGCAAGGTATAGAGGTTGGAAATCGAGGGTAAATTGTTACTGCTATCG<br>GTGTTACTGGAAAGAACATGCTATCCATTAGCAGAAAAGTGGAAAGGGTAAACTCCACAAGAATTAAACAGATT<br>CAGTTGAATTTCAGAGATGTTGTTACTGGCATTGCAAAAGTTACTAGAGTTACAATGATCTGCCATTGACAGG<br>TAAACAATCTGAAAAGGTTGCTGAAATTGTTGTCACATTGAAAGGTATTGGTACTTACACAGATGATGAAGG<br>TAGAGCTATGCAAAAGTTGGATGTTTGTGTTAGATCAGAAACATTCCACCAAGGTGCTATCATGTTCACTCA<br>CCATTAGTTGTTGACAATTCTTGTCAAAGATGTTGACTGGTACAGCTAACCGCAGTTATTGAAAGAAC<br>AATTGCTGAAGCTGTTGAAATCAATCATGGTAAACATGGTCTCCAGCTGCAAAATGTTAGTGTGTT<br>AGTTGCGAGAATTGTTGAAAGAACATTGCTGAAGAAGCATCAGTTGGTAAACCAACTGAAAGTAA<br>CCAGTTATTGGTGTAA  |
| <i>PsCHI</i> | ATGGCAAAACCACCATCTGTTCAAGGTGTTAACATGCAATCTTATGCTTTCAGCAACAGTTAACCCACAGGTTCTA<br>CTAAACATTGTTTAGGGTGCAGGGTTAGAGGTTGGAAAGTCCAACTGGTCAATTGTTACTGCTAT<br>CGGTGTTACTGGAAAGATAACGCAATCACTTCAATTGCTGTTAGTGGAAAGGTTAAACTGCTGAAGAATTAA<br>ATCTGATGATTCTTGTGTTAGAGATCAGTTACAGGTCCATTGAAAGTAAACTCAAGTTACAATGATCTGCCATTGACT   |

---

GGTCAACAATACTCAGAAAAGGTTACAGAAAACGTGTTGCCTACTGGAAAGCAGTTGGTGCCTAACGTGCTGAA  
 GCATCTGCTATTGAAAAGTTATTGAAGTTAAAGATGAAAAGTTCCACCAGGTTCTCAATTGGTTACTCAAAC  
 ACCAGAAGGTTCTTAACATCGGTTTCAAGGATGGTGGTGCAGAAGTGGTAATGCAGTTGAAATAA  
 GCAATTGTCGAAGCTGTTAGAATCAATCATCGAACATGGTTCTCAGAAGCTAACATCAGTGA  
 AGAATTCTGAATTGTTAAATAA  


---

*SmCHI*

ATGGCTGCTTAACTGCAATCGAAAACACGTTTCCACCAACTATGGTAAGCCAGTTGGTCTAACAA  
 CTTCTTTGGCTGGTCAGGTTCAAGAGGTTAGAAATCGAAGGTTAGATTGCTTAAGTTACTGCTATCGCAGTTA  
 CTTGGAAGAATCTGCTATTCCATTAGCTGCAAAGTGGAAAGGTTAATCTCAGAAGAATTAACGATTGATTGAA  
 TTTTCAAGGATATCGTACAGGTCCATTGCAAAGGTTACTCAAGTTAACATGATCTGCCATTGACTGGTAAACAA  
 ACTCTGAAAAGGTTGCTGAAAACGTGTTGCTAACGTTGAAAGCAATTGGTACTTACTCTGATGCTGAATCACAAGCAA  
 TCAGAAAAGTCTGAAACGTTTCAATCTGAAACATCCCACATGGTCATCAATTGTTACTCAATCTCATTGG  
 TTCATTAACAATTCTTTCAAGGATGATTGTTCCATCAATGGTAACGCTGTTATTGAAATAAGCAATTGCTG  
 AAGCAGTTAGATTCAATCATCGTAAACATGGTGGTCCAGCTGCAAAGTGTCAATCGTAAGAGAGTTCTG  
 AATTGTTAGAAAATCAAATGCTGAAGCAGTTGTAATGAAAAACAGGTTCTGGTTCAACAAATTCAATAA  


---

ATGTCTAATTTGTTGATGCAATGAGAGCTGAGCTCCTGGTAACTGCTCCTTATTAGAATCGATAACACTAGAA  
 CATGGACTTACGATGATGCAATTGCTTATCTGGTAAATTGATCAGCTATGGATGCTTGGGATTAGACCAGGT  
 ACAGAGTGTGTTCAAGTTGAAAATCTGAGCAGCTTGTATCTGGTAACTTGTGAGATCAGGTGCTGTT  
 TTTGCCATTGAAACAGCATACACTTGCTGAAATTGAGATTACTTCATCGGTGACGAGAAGGTTGCTATTGTTGAA  
 TGCTTCTCAGCAAGAGCTGGTGTAAACTATTGCAAACCCAAGAGGTTGCTATTGTTGAAACATTAGATGCA  
 TTGTTGTTCAATTGTTAGATTGGCAAGAGATGAACCAAGCTGATTTGTTGATGCACTAGATCAGCTGATTTGG  
 GCTATTGTAACACTTGTTACTACAGGAGATCAAAGGTCAGTTGACATGGTAACTTGTGAAACGCTT  
 TGACTTGGAGAGATTGGAGAGTTACAGCAGGTGACAGATTGATCCATGCTTGGCAATCTCCACTCATGG  
 ATTGTTGCTACAAACGTTACTTGTTAGCAGGTGCTCTATGTTTGTGCAAGTTCAGTCCAGAAGAAATCTG  
 TCTTGATGCCACAAGCTACTATGTTGATGGGTGTTCAACATTACGTTAGATTGTTGCAATCAGCAAGATTGG  
 AGCAAGCAGTTGCTAACATCAGATTGTTATTCTGGTCAGCACATTGTTAGCTGAAACACATACTGAATTCAAGC  
 AAGAACTGGTCATGCTATTAGAAAGATACGGTATGACAGAAAACATAACATGAACACTCTAACCCATACGA  
 AGGTAAGAATTGCTGGTACAGTTGGTTCCATTGCCAGATGTTACAGTTAGGTTACTGATCCAGCAACAG  
 GTTGGTTAGTTGAGAGATTGGGAGAGTTACAGCAGGTGACAGATTGATCCATGCTTGGCAATCTCCACT  
 ATTGTTGCTACAAACGTTACTTGTTAGCAGGTGCTCTATGTTTGTGCAAGTTCAGTCCAGATTGTTG  
 GAAACCAAGGTGCAAGTTGGATGAAAAGCAATGTTCTGCTTGCAGATAGTTGCAAGATACAAGCA  
 AAGAGAATCATCTCGTCAAGATTGCAAGAAATACAATGGTAAAGTCAAAAGAATCTTGAGACAACAATATG  
 CTGATTGACACAAGAAACTAA  


---

*RtmatB*

ATGGGTATGAAATTGTTGCTATGGTTGTTGATTGCTATGTCATCGCAACTATCCAACCAATTATGGG  
 CTTTGGCATTGCTGGTCATTGTTAGGTTCTATGATCATCGGTATGAAAGACAAACGAAATCTCGCTGGTT  
 ATCAGATTGTTTGTGACTTGGTGCAGTTACATATTGTCGCTATCGCACAAATTATGGTACTATCGATTGG  
 GTTGAATGTCGTTGATTAGTTAGAGGTTAGATTGTTGACTGGTAAATCGATAGAGATGGTACGTT  
 CAGGTTGGTGCCTTGGTCCAGCTGAGTTGCTATTGGCACCAGTTGCTTGTCTTCAGTTCAATACAGA  
 TCCATCCAGTTGATGGTTAATGGTATTCTGGTCAGGGTTCTCAAGCAGGTTACATTGTC  
 TATCACTAACCAATCGTGTAAAGCAGGTTACATTGTC  
 GGCTATCGAGTTGGTTCTTGGTGTCTGGTCTAGAGTTGAAACATGATCCAGCATATTGGTC  
 CCAGAATTGCACTCAGAAGGTTCTGGTCAATTAGAGGTTGCTTGTACTCCAGCTAACCAATTAGAGA  
 GCATACGGTACTGCTGCAGATAACAGTACTACATTGAGATTGAAACACGAAAGAATCACTACATTG  
 CGTTAGGTATGGTGCATTGGTTTAAGTTAATGTTGTTAGTTGCAATGACTGTTGCTGTTGG  
 TATCTCCAAAGACACAAAGGCTGCAATCGATAAGTTCTGGTCAACTGTTGATCGCTGG  
 CGTTGGTTATGGAAAAGCTGGTACTGTTGATTGCTTCAAGGGTATTCTCATTGGG  
 GCTTTGGTTGTTGTTCACTGGTCAATTGTTCAGCTTGCATCTCAACAGCTTATTGGG  
 TGAATTGTTGATCTTCAACAAATGGTCTTGGTCAATTGTTGCTAATGCA  
 CAATTGTTGATCTTCAACAAATGGTCTTGGTCAATTGTTGCTTGGTTGGTTGG  
 TTTAA  


---

*RtmatC*

ATGGGTATGAAATTGTTGCTATGGTTGTTGATTGCTATGTCATCGCAACTATCCAACCAATTATGGG  
 CTTTGGCATTGCTGGTCATTGTTAGGTTCTATGATCATCGGTATGAAAGACAAACGAAATCTCGCTGGTT  
 ATCAGATTGTTTGTGACTTGGTGCAGTTACATATTGTCGCTATCGCACAAATTATGGTACTATCGATTGG  
 GTTGAATGTCGTTGATTAGTTAGAGGTTAGATTGTTGACTGGTAAATCGATAGAGATGGTACGTT  
 CAGGTTGGTGCCTTGGTCCAGCTGAGTTGCTATTGGCACCAGTTGCTTGTCTTCAGTTCAATACAGA  
 TCCATCCAGTTGATGGTTAATGGTATTCTGGTCAGGGTTCTCAAGCAGGTTCT  
 TATCACTAACCAATCGTGTAAAGCAGGTTACATTGTC  
 GGCTATCGAGTTGGTTCTTGGTGTCTGGTCTAGAGTTGAAACATGATCCAGCATATTGGTC  
 CCAGAATTGCACTCAGAAGGTTCTGGTCAATTAGAGGTTGCTTGTACTCCAGCTAACCAATTAGAGA  
 GCATACGGTACTGCTGCAGATAACAGTACTACATTGAGATTGAAACACGAAAGAATCACTACATTG  
 CGTTAGGTATGGTGCATTGGTTTAAGTTAATGTTGTTAGTTGCAATGACTGTTGCTGTTGG  
 TATCTCCAAAGACACAAAGGCTGCAATCGATAAGTTCTGGTCAACTGTTGATCGCTGG  
 CGTTGGTTATGGAAAAGCTGGTACTGTTGATTGCTTCAAGGGTATTCTCATTGGG  
 GCTTTGGTTGTTGTTCACTGGTCAATTGTTCAGCTTGCATCTCAACAGCTTATTGGG  
 TGAATTGTTGATCTTCAACAAATGGTCTTGGTCAATTGTTGCTAATGCA  
 CAATTGTTGATCTTCAACAAATGGTCTTGGTCAATTGTTGCTTGGTTGGTTGG  
 TTTAA

---

**Supplementary Table 5. Expression modules in the integration constructs used in this study**

| ID  | DNA fragment <sup>a</sup>   |
|-----|---|
| M1  | <u>XII-4 up-TDH3p-At4CL1-ADH1t +TDH2t</u>   |
| M2  | <u>ADH1t +TDH2t-HaCHS-CCW12p+tHXT7p-PhCHI-FBAt-XII-4 dn</u>                             |
| M3  | <u>ADH1t +TDH2t-HaCHS-CCW12p+tHXT7p-PsCHI-FBAt-XII-4 dn</u>                             |
| M4  | <u>ADH1t +TDH2t-HaCHS-CCW12p+tHXT7p-SmCHI-FBAt-XII-4 dn</u>                             |
| M5  | <u>ADH1t +TDH2t-RsCHS-CCW12p+tHXT7p-PhCHI-FBAt-XII-4 dn</u>                             |
| M6  | <u>ADH1t +TDH2t-RsCHS-CCW12p+tHXT7p-PsCHI-FBAt-XII-4 dn</u>                             |
| M7  | <u>ADH1t +TDH2t-RsCHS-CCW12p+tHXT7p-SmCHI-FBAt-XII-4 dn</u>                             |
| M8  | <u>ADH1t +TDH2t-SmCHS-CCW12p+tHXT7p-PhCHI-FBAt-XII-4 dn</u>                             |
| M9  | <u>ADH1t +TDH2t-SmCHS-CCW12p+tHXT7p-PsCHI-FBAt-XII-4 dn</u>                             |
| M10 | <u>ADH1t +TDH2t-SmCHS-CCW12p+tHXT7p-SmCHI-FBAt-XII-4 dn</u>                             |
| M11 | <u>X-3 up-TPI1p-EcaroL-pYX212t-ADH1t-ARO7 G141S-TEF1p-PGK1p-ARO4 K229L-CYC1t-X-3 dn</u> |
| M12 | <u>X-4 up-CYC1t-ARO1-TPI1p-TDH3p-ARO2-ADH1t-TDH2t-ARO3-TEF1p-X-4 dn</u>                 |
| M13 | <u>X-2 up-GPM1p-PHA2-CYC1t-X-2 dn</u>   |
| M14 | <u>XII-1 up-TDH3p-At4CL1-ADH1t-XII-1 dn</u>   |
| M15 | <u>XII-1 up-TDH2t-RsCHS-CCW12p-tHXT7p-PsCHI-FBAt-XII-1 dn</u>                           |
| M16 | <u>XII-1 up-TDH3p-At4CL1-ADH1t-TDH2t</u>  |
| M17 | <u>ADH1t-TDH2t-RsCHS-CCW12p-tHXT7p-PsCHI-FBAt-XII-1 dn</u>                              |
| M18 | <u>XII-5 up-pYX212t-PsCHI-PGKp-TEF1p-RsCHS-FBAt-CYC1t</u>                               |
| M19 | <u>FBAt-CYC1t-At4CL1-TPI1p-XII-5 dn</u>   |
| M20 | <u>XII-5 up-CYC1t-At4CL1-TPI1p-XII-5 dn</u>   |
| M21 | <u>XII-5 up-TEF1p-RsCHS-FBAt-XII-5 dn</u>   |
| M22 | <u>XII-5 up-pYX212t-PsCHI-PGKp-XII-5 dn</u>   |
| M23 | <u>XII-5 up-pYX212t-PsCHI-PGKp-TEF1p-RsCHS-FBAt-XII-5 dn</u>                            |
| M24 | <u>XI-1 up-pYX212t-PsCHI-PGKp-TEF1p-RsCHS-FBAt-CYC1t</u>                                |
| M25 | <u>FBAt-CYC1t-At4CL1-TPI1p-XI-1 dn</u>  |
| M26 | <u>XI-1 up-CYC1t-At4CL1-TPI1p-XI-1 dn</u>   |
| M27 | <u>XI-1 up-TEF1p-RsCHS-FBAt-XI-1 dn</u>   |
| M28 | <u>XI-1 up-pYX212t-PsCHI-PGKp-XI-1 dn</u>   |
| M29 | <u>XI-1 up-pYX212t-PsCHI-PGKp-TEF1p-RsCHS-FBAt-XI-1 dn</u>                              |
| M30 | <u>XI-3 up-TDH2t-RsCHS-CCW12p-tHXT7p-PsCHI-FBAt-XI-3 dn</u>                             |
| M31 | <u>XI-2 up-TPI1p-ACC1 S659A,S1157A up 500bp</u>   |
| M32 | <u>ACC1 S659A,S1157A</u>  |
| M33 | <u>ACC1 S659A,S1157A dn 500bp-TDH2t-XI-2 dn</u>   |
| M34 | <u>XII-3 up-TDH2t-matB-TDH3p-tHXT7p</u>   |
| M35 | <u>TDH3p-tHXT7p-matC-CYC1t-XII-3 dn</u>   |
| M36 | <u>X-2 up-TEF1-NLS_FapR-ADH1t-X-2 dn</u>  |
| M37 | <u>XI-3 up-GPM1p-AtPAL2-FBA1t-XI-3 dn</u>   |
| M38 | <u>XI-3 up-CCW12p-AtPAL2-FBA1t-XI-3 dn</u>  |
| M39 | <u>XI-3 up-CCW12p_BS2-AtPAL2-FBA1t-XI-3 dn</u>  |
| M40 | <u>XI-3 up-CCW12p_BS123-AtPAL2-FBA1t-XI-3 dn</u>  |
| M41 | <u>XI-3 up-TDH3p-AtPAL2-FBA1t-XI-3 dn</u>   |
| M42 | <u>XI-3 up-TDH3p_BS23-AtPAL2-FBA1t-XI-3 dn</u>  |
| M43 | <u>XI-3 up-TEF1p-AtPAL2-FBA1t-XI-3 dn</u>   |
| M44 | <u>XI-3 up-TEF1p_BS123-AtPAL2-FBA1t-XI-3 dn</u>   |
| M45 | <u>FDC1 up-pYX212t-DCR1-TDH3p-tHXT7p</u>  |
| M46 | <u>TDH3p-tHXT7p-AGO1-CYC1t-FDC1 dn</u>  |
| M47 | <u>XI-5 up-TDH3p-FAS1_sense 200bp-rad9_intron1 80bp</u>                                 |
| M48 | <u>XI-5 up-TDH3p-FAS1_sense 250bp-rad9_intron1 80bp</u>                                 |

|     |  |
|-----|--|
| M49 | <i>XI-5 up-TDH3_BS23p-FAS1_sense 200bp-rad9_intron1 80bp</i>       |
| M50 | <i>XI-5 up-TDH3_BS23p-FAS1_sense 250bp-rad9_intron1 80bp</i>       |
| M51 | <i>rad9_intron1 80bp-FAS1_antisense 200bp-TDH2t-<u>XI-5 dn</u></i> |
| M52 | <i>rad9_intron1 80bp-FAS1_antisense 250bp-TDH2t-<u>XI-5 dn</u></i> |

<sup>a</sup>Bold font indicates genes expressed; *p*, indicates promoter; *t*, indicates terminator; underline, indicates up-stream (up) and down-stream (dn) sequences for Cas9-mediated homologous recombination.

## Reference

1. Mans R, *et al.* (2015) CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 15(2).
2. Liu Q, *et al.* (2019) Rewiring carbon metabolism in yeast for high level production of aromatic chemicals. *Nat Commun* 10(1):4976.
3. Rodriguez A, Kildegard KR, Li M, Borodina I, & Nielsen J (2015) Establishment of a yeast platform strain for production of p-coumaric acid through metabolic engineering of aromatic amino acid biosynthesis. *Metab Eng* 31:181-188.
4. Li M, *et al.* (2015) De novo production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. *Metab Eng* 32:1-11.
5. Shi S, Chen Y, Siewers V, & Nielsen J (2014) Improving production of malonyl coenzyme A-derived metabolites by abolishing Snf1-dependent regulation of Acc1. *mBio* 5(3):e01130-01114.
6. David F, Nielsen J, & Siewers V (2016) Flux Control at the Malonyl-CoA Node through Hierarchical Dynamic Pathway Regulation in *Saccharomyces cerevisiae*. *ACS Synth Biol* 5(3):224-233.
7. Dabirian Y, *et al.* (2019) Expanding the Dynamic Range of a Transcription Factor-Based Biosensor in *Saccharomyces cerevisiae*. *ACS Synth Biol* 8(9):1968-1975.
8. Wang G, *et al.* (2019) RNAi expression tuning, microfluidic screening, and genome recombineering for improved protein production in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 116(19):9324-9332.