

Table S1. *S. cerevisiae* strains used in this study

Strain	Description	Reference or source
W1 to W121	<i>S. cerevisiae</i> strain collection from industry, lab and nature	Our lab collection
ScY01	Diploid strain, evolved thermotolerant strain derived from Ethanol Red	[11]
W65	Diploid strain, thermosensitive strain	Our lab collection
ScY01 α	Haploid segregant from ScY01 with similar thermotolerance, <i>MATα</i>	This study
W65a	Haploid segregant from W65 with similar thermosensitivity, <i>MATα</i>	This study
ScY01 α -tp	Haploid segregant from ScY01 with similar thermotolerance, <i>MATα</i> , <i>ho::BleoR</i>	This study
W65a-sp	Haploid segregant from W65 with similar thermosensitivity, <i>MATα</i> , <i>ho::KanMX4</i>	This study
tpxsp	Hybrid diploid strain obtained by crossing ScY01 α -tp and W65a-sp	This study
tp- <i>rxl2</i> Δ xsp	Hybrid diploid strain, ScY01 α -tp (<i>rxl2::hphB</i>) crossed with W65a-sp	This study
tpxsp- <i>rxl2</i> Δ	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp (<i>rxl2::hphB</i>)	This study
tp- <i>vid24</i> Δ xsp	Hybrid diploid strain, ScY01 α -tp (<i>vid24::hphB</i>) crossed with W65a-sp	This study
tpxsp- <i>vid24</i> Δ	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp (<i>vid24::hphB</i>)	This study
tp- <i>ecm22</i> Δ xsp	Hybrid diploid strain, ScY01 α -tp (<i>ecm22::hphB</i>) crossed with W65a-sp	This study
tpxsp- <i>ecm22</i> Δ	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp (<i>ecm22::hphB</i>)	This study
tp- <i>vps34</i> ^{E591D} xsp	Hybrid diploid strain, ScY01 α -tp containing the <i>vps34</i> ^{E591D} allele from W65a-sp crossed with W65a-sp	
tpxsp- <i>vps34</i> ^{D591E}	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp containing the <i>vps34</i> ^{D591E} allele from ScY01 α -tp	This study
tp- <i>csc1</i> ^{K376Q} xsp	Hybrid diploid strain, ScY01 α -tp containing the <i>csc1</i> ^{K376Q} allele from W65a-sp crossed with sp	This study
tpxsp- <i>csc1</i> ^{Q376K}	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp containing the <i>csc1</i> ^{Q376K} allele from ScY01 α -tp	This study
tp- <i>ira2</i> Δ xsp	Hybrid diploid strain, ScY01 α -tp (<i>ira2::hphB</i>) crossed with W65a-sp	This study
tpxsp- <i>ira2</i> Δ	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp (<i>ira2::hphB</i>)	This study
tp- <i>avo1</i> ^{V853A} xsp	Hybrid diploid strain, ScY01 α -tp containing the <i>avo1</i> ^{V853A} allele from W65a-sp crossed with W65a-sp	This study
tpxsp- <i>avo1</i> ^{A853V}	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp containing the <i>avo1</i> ^{A853V} allele from ScY01 α -tp	This study
tp- <i>dap1</i> Δ xsp	Hybrid diploid strain, ScY01 α -tp (<i>dap1::hphB</i>) crossed with W65a-sp	This study
tpxsp- <i>dap1</i> Δ	Hybrid diploid strain, ScY01 α -tp crossed with W65a-sp (<i>dap1::hphB</i>)	This study
Plasmids		
pREMI-Z	Zeocin-resistance plasmid containing zeocin resistance cassette (BleoR)	[46]
pFA6-kanMX4	Geneticin-resistance plasmid containing KanMX4 module	[47]
pRS426-hphB	Hygromycin-resistance plasmid by replacing <i>URA3</i> coding region with the hygromycin B resistance gene from <i>Escherichia coli</i>	In our lab, [56]

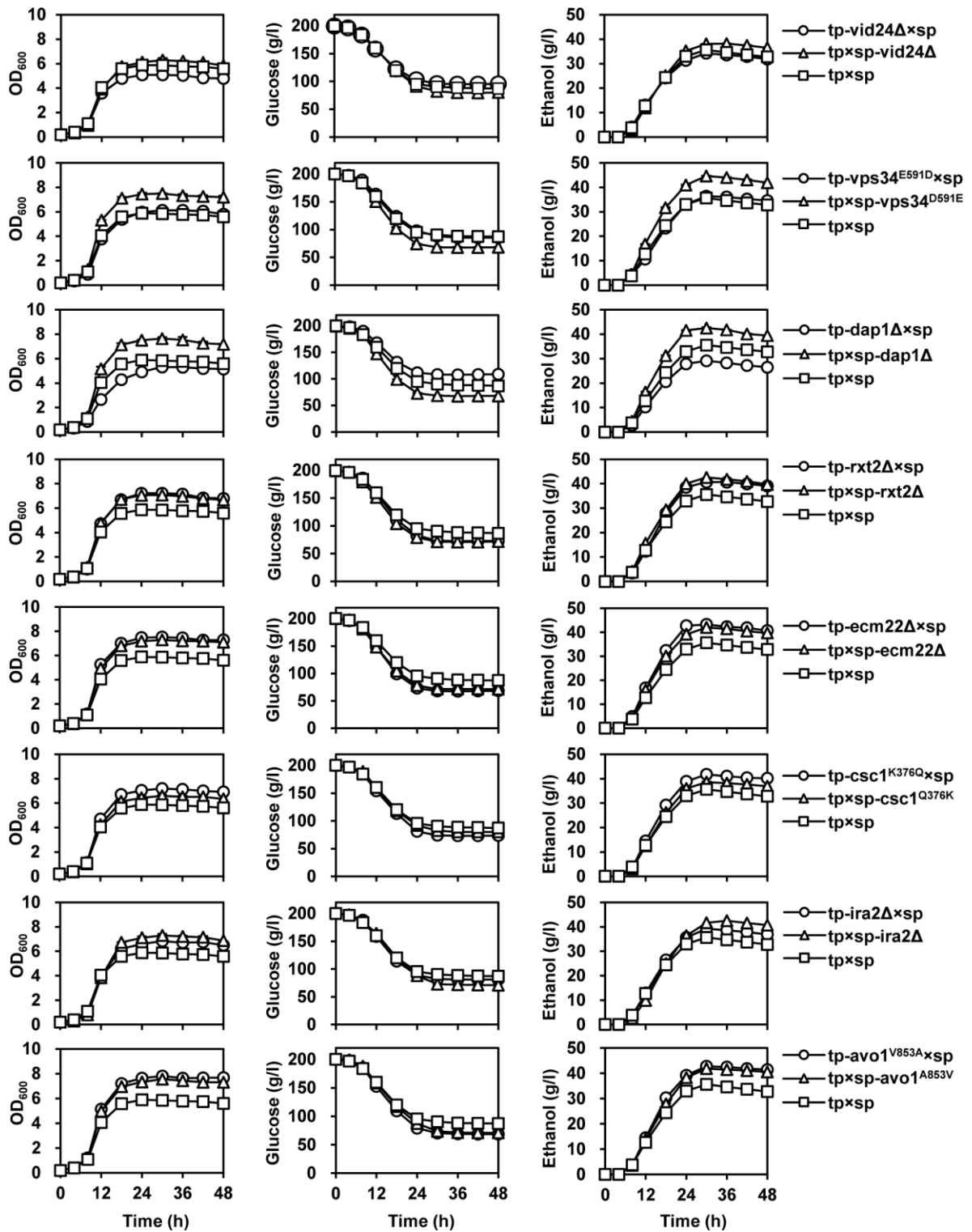


Figure S1. Fermentation profiles of RHA and allele replacement strains of the causative genes.

The RHA and allele replacement strains were detailed in Additional file 1: Table S1. High-temperature fermentation capacities were evaluated at 42°C in 100-

ml Erlenmeyer flasks containing 50 ml YP medium with 200 g/l glucose at 220 rpm. Data represent the mean and standard error of duplicate cultures at each condition (some error bars are covered by symbols).