Supplemental Materials

Casein Kinase I Isoform Hrr25 Is a Negative Regulator of Haa1 in the Weak Acid Stress Response Pathway in *Saccharomyces cerevisiae*

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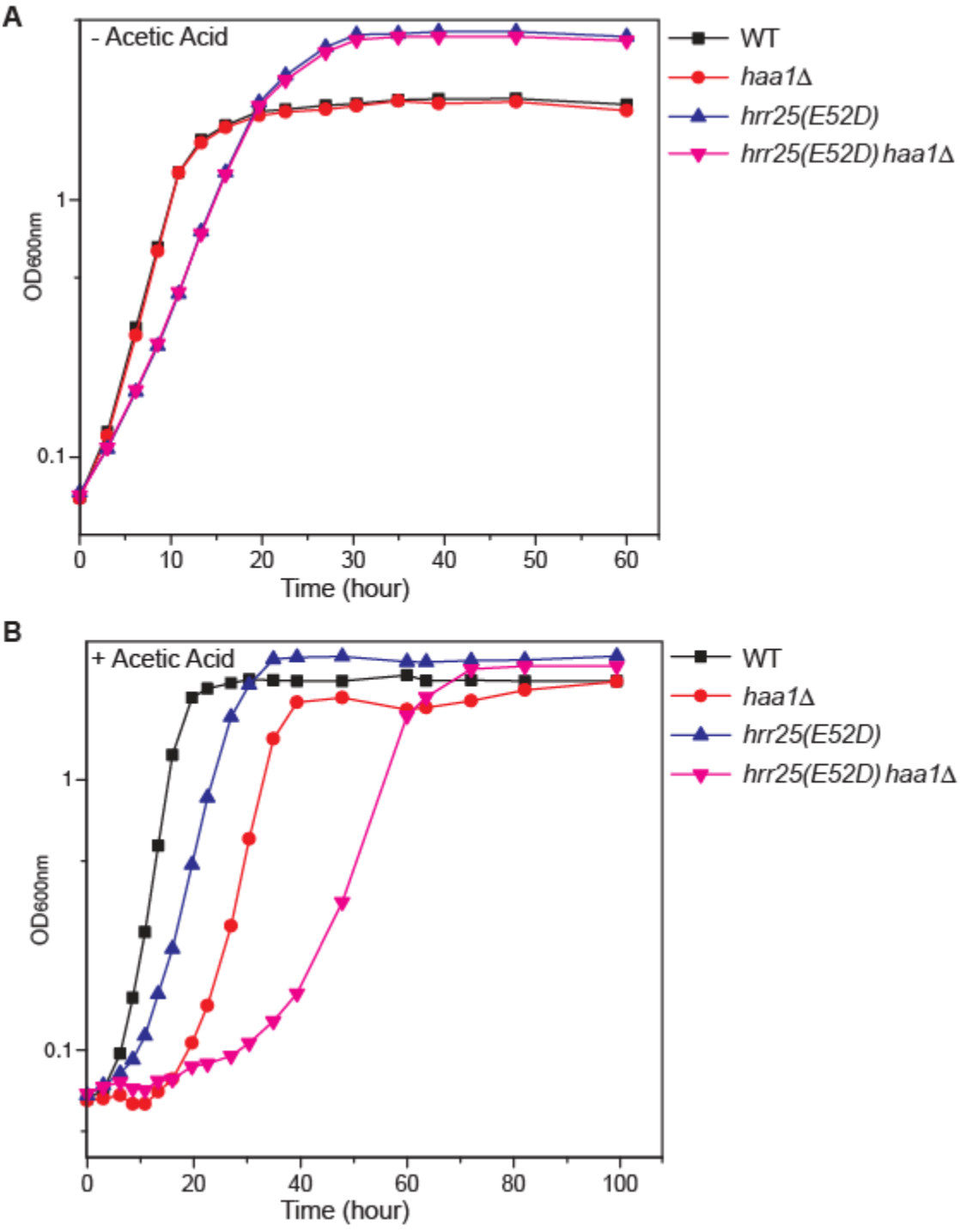
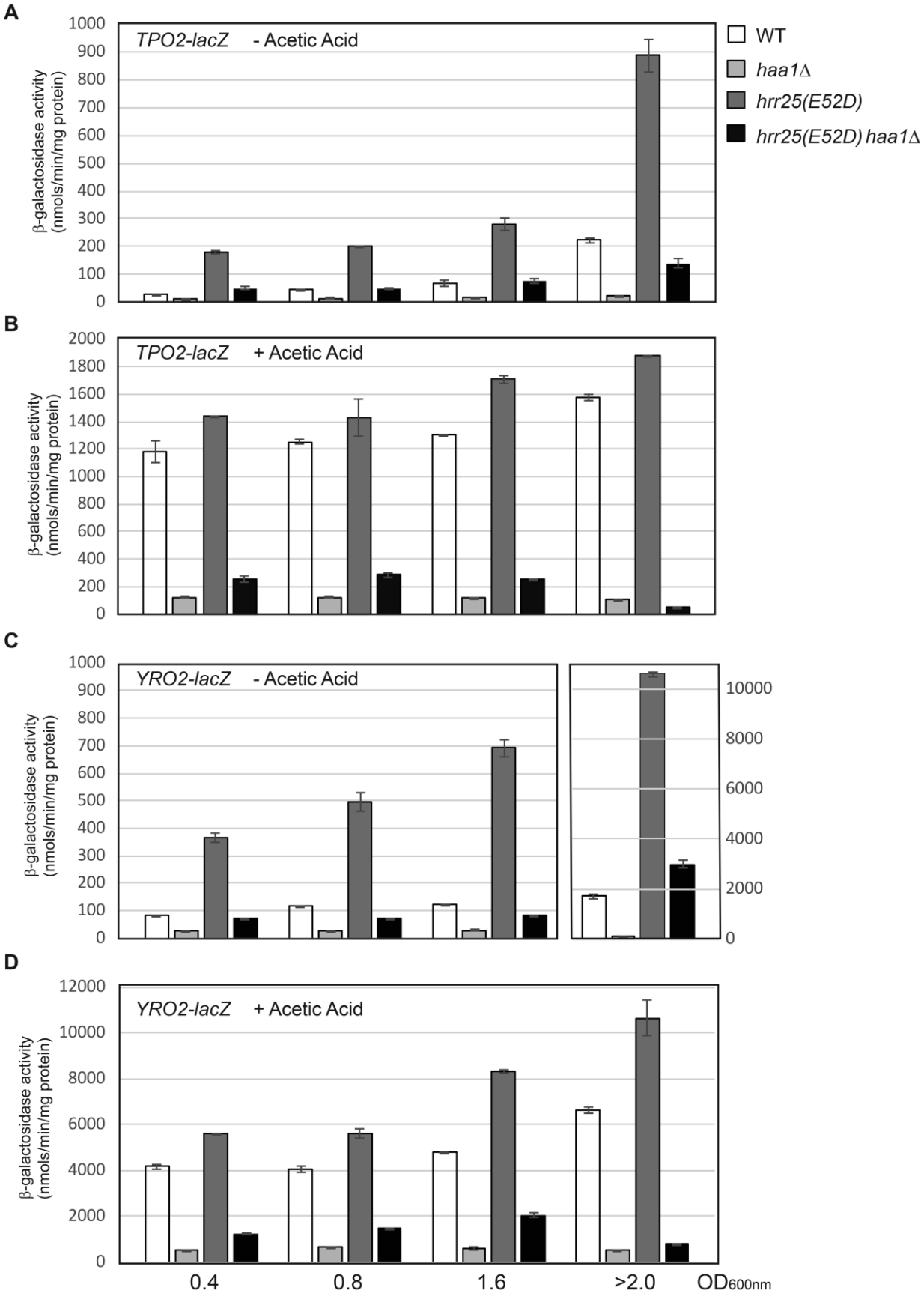


Supplemental Figure S1. The growth curve of wild-type (WT, BY4741), *haa1Δ* (ZLY4043), *hrr25(E52D)* (ZLY4467), *hrr25(E52D) haa1Δ* (ZLY4637) mutant cells grown in MM4 medium (supplemented with uracil, leucine, histidine, and methionine at standard concentrations) without (panel A) and with 60 mM acetic acid (panel B). The higher OD values of stationary phase cultures of *hrr25(E52D)* and *hrr25(E52D) haa1Δ* mutant strains are likely to result from the Leu^+ phenotype of these two mutant strains, which carry a pRS415 plasmid encoding the *hrr25(E52D)* mutant allele. Each growth curve is representative of results from two independent experiments.


A
TPO2-lacZ - Acetic Acid
β-galactosidase activity (nmols/min/mg protein)
WT
haa1Δ
hrr25(E52D)
hrr25(E52D) haa1Δ
B
TPO2-lacZ + Acetic Acid
β-galactosidase activity (nmols/min/mg protein)
C
YRO2-lacZ - Acetic Acid
β-galactosidase activity (nmols/min/mg protein)
D
YRO2-lacZ + Acetic Acid
β-galactosidase activity (nmols/min/mg protein)
0.4
0.8
1.6
>2.0
OD600nm

Supplemental Figure S2. Increased expression of *TPO2-lacZ* and *YRO2-lacZ* reporter genes due to an *hrr25(E52D)* mutation requires Haa1. Wild-type (BY4741) and isogenic *haa1Δ* (ZLY4043), *hrr25(E52D)* (ZLY4467) and *hrr25(E52D) haa1Δ* double mutant (ZLY4637) strains carrying centromeric plasmids encoding a *TPO2-lacZ* (pZL3158) or *YRO2-lacZ* (pZL3164) reporter gene were grown in MM4 medium without (panel A) and with 60 mM acetic acid (panel B) for six generations to reach OD_{600nm} 0.4. Three aliquots of each culture were then collected at OD_{600nm} 0.4, 0.8, 1.6. and > 2.0 (stationary phase). β-galactosidase activity assays were conducted as described in the main text. Figure S2C also shows that the expression of *YRO2-lacZ* is highly induced in stationary phase, which is consistent with published findings (1).

References

1. **Takabatake A, Kawazoe N, Izawa S.** 2015. Plasma membrane proteins Yro2 and Mrh1 are required for acetic acid tolerance in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol **99:**2805-2814.