

Supporting Information

Zhang et al. 10.1073/pnas.1405204111

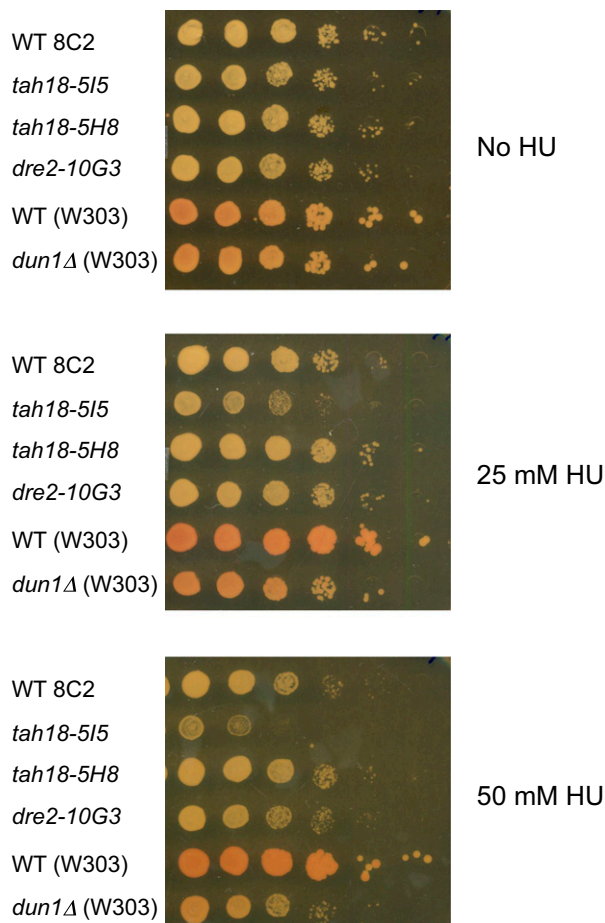


Fig. S1. The original *tah18-5I5* temperature-sensitive (*ts*) mutant is sensitive to hydroxyurea (HU). WT and mutant cells from log-phase cultures were dot-plated in 10-fold serial dilution, starting at 10^6 cells, on yeast extract/peptone/dextrose (YPD) plates or on YPD plates containing 25 mM or 50 mM HU and were incubated at 23 °C for 3 d before being imaged.

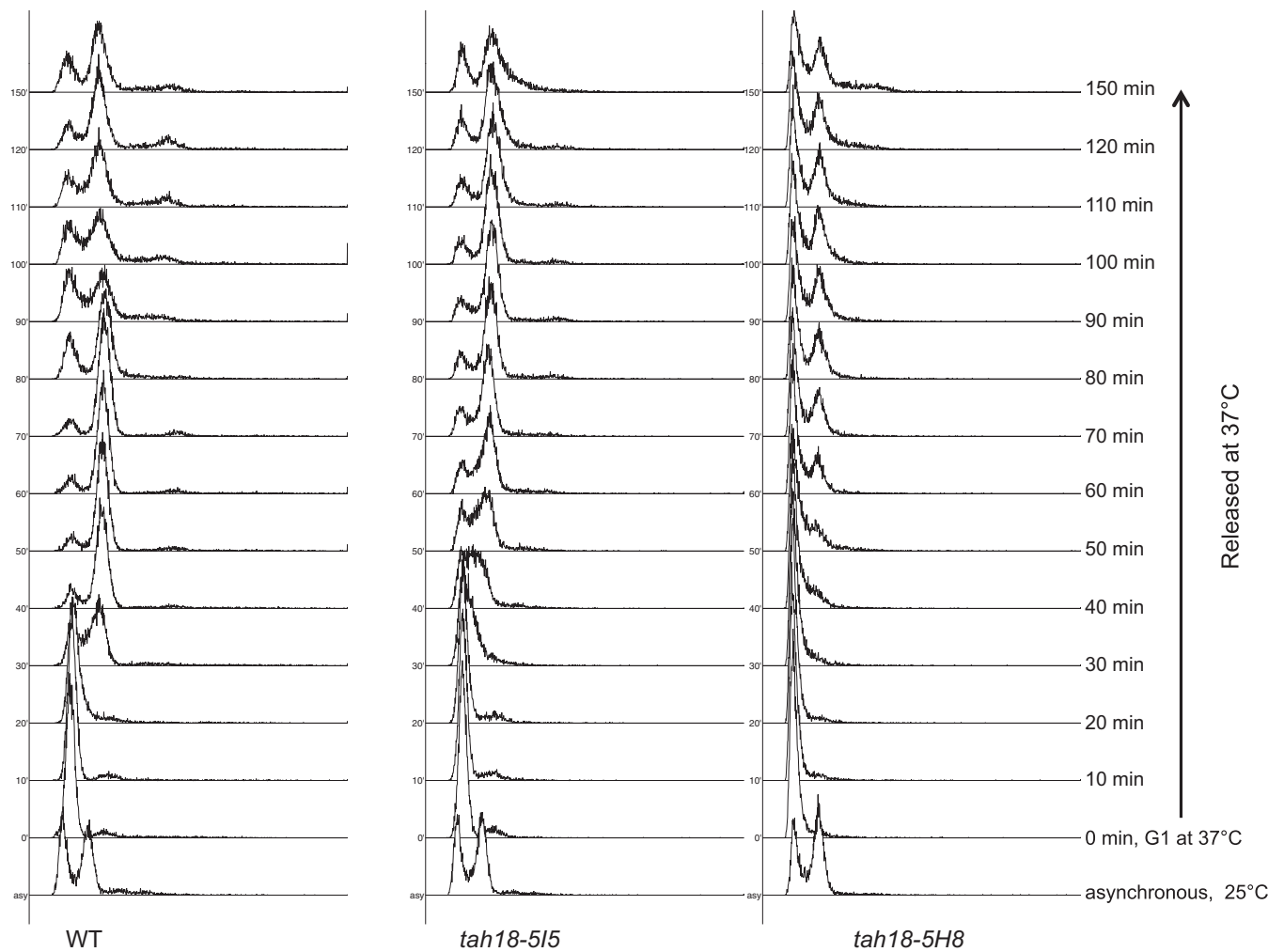


Fig. S2. Defects in cell-cycle progression in the original *tah18* ts mutant strains. WT, *tah18-5I5*, and *tah18-5H8* mutant cells from log-phase cultures were synchronized in G1 phase by α factor-mediated arrest at 23 °C before being shifted to 37 °C for 1 h. Cells were released into the cell cycle by washing off the α factor, and samples were taken at 10-min intervals and processed for flow cytometry.

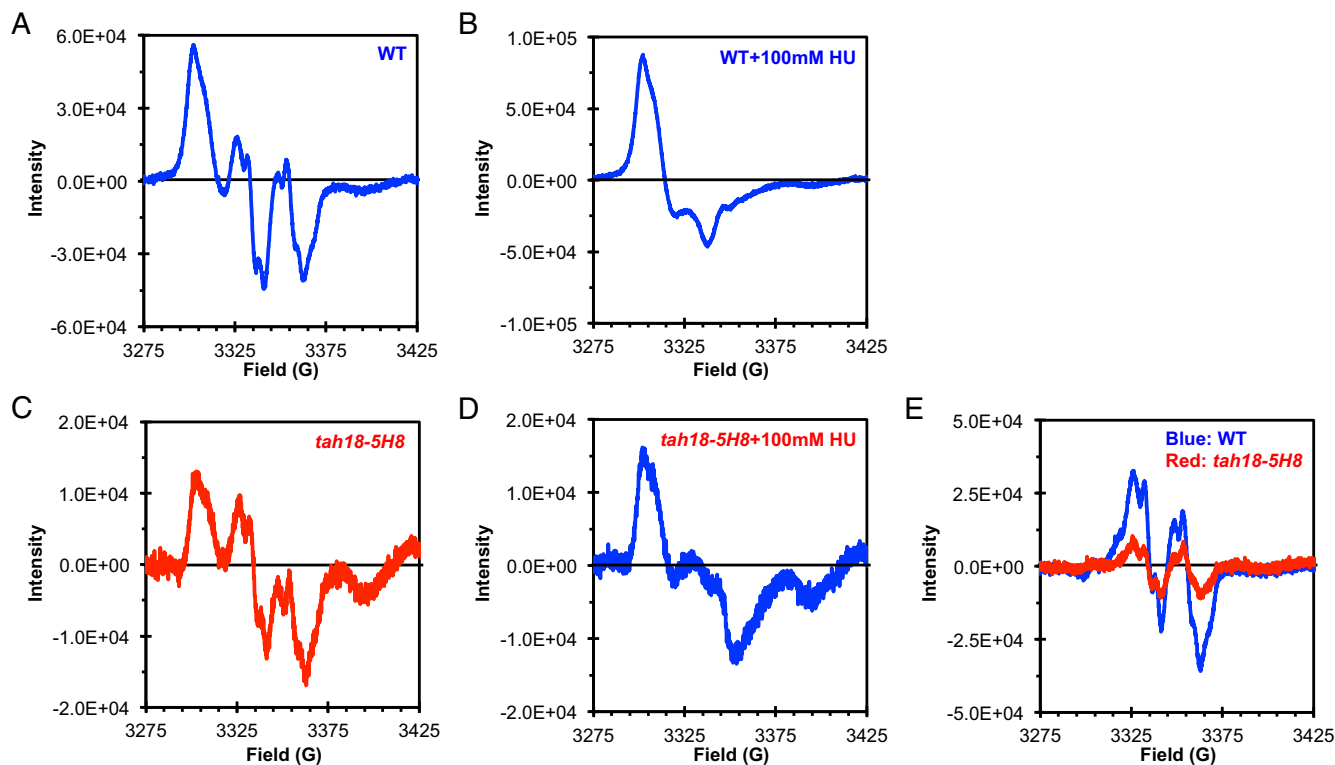


Fig. S3. Comparison of tyrosyl radical ($Y\bullet$) content in the original *tah18-5H8* mutant and its isogenic WT strain. (A and B) EPR spectra of the WT cells without HU treatment (Left) or after incubation with 100 mM HU for 2 h (Right). (C and D) EPR spectra of the *tah18-5H8* cells without HU treatment (Left) or after incubation with 100 mM HU for 2 h (Right). (E) Comparison of WT (blue) and *tah18-5H8* (red) EPR signals after the HU-treated spectra were subtracted from the untreated spectra.

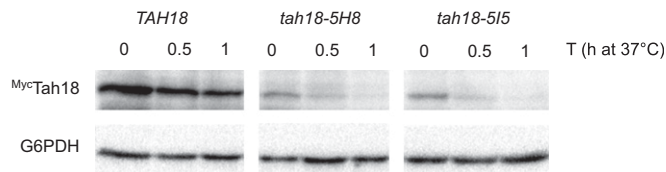


Fig. S4. Instability of the Tah18-ts mutant proteins at the nonpermissive temperature. The $\Delta tah18$ mutant harboring a centromeric plasmid (one to three copies per cell) that expressed N-terminally Myc3-tagged *TAH18*, *tah18-5H8*, or *tah18-5I5* was grown at 23 °C to log phase and shifted to 37 °C at time 0. Cells were harvested at 0.5 and 1 h after temperature shift, and the Myc3-Tah18 protein levels were monitored by Western blotting with the 9E10 monoclonal anti-Myc antibody. G6PDH was probed on the same blot as a loading control.

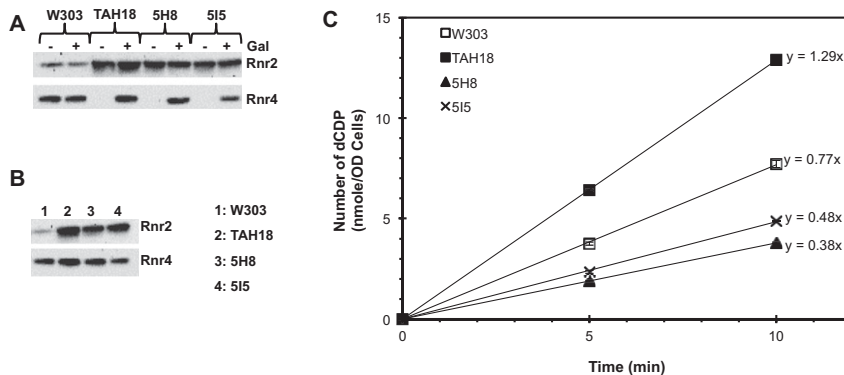


Fig. S5. Comparison of protein levels and activities of $\beta\beta'$ in $\Delta crt1 GalRNR4 TAH18$ and $\Delta crt1 GalRNR4 tah18ts$ mutant cells before and after *GalRNR4* induction. (A) Comparison of Rnr2 (β) and Rnr4 (β') protein levels in W303, $\Delta crt1 GalRNR4 TAH18$ (*TAH18*), $\Delta crt1 GalRNR4 tah18-5H8$ (*5H8*), and $\Delta crt1 GalRNR4 tah18-5I5$ (*5I5*) cells before (-) and 2 h after (+) β' induction. (B) Comparison of β and β' protein levels at the 2-h time point after β' induction. (C) Assays of $\beta\beta'$ activities at the 2-h time point after β' induction.

Table S1. Yeast strains used in this study

Strain	Genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	(1)
BY4742	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	(1)
<i>GalRNR4</i>	BY4741, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4</i>	(2)
<i>Δaft1</i>	BY4741, <i>aft1::KanMX4</i>	EUROSCARF
<i>Δaft2</i>	BY4741, <i>aft2::KanMX4</i>	EUROSCARF
<i>Δcrt1</i>	BY4741, <i>crt1::KanMX4</i>	EUROSCARF
<i>Δpmr1</i>	BY4741, <i>pmr1::KanMX4</i>	EUROSCARF
<i>Δdun1</i>	BY4741, <i>dun1::KanMX4</i>	EUROSCARF
<i>Δgrx3Δgrx4</i>	BY4742, <i>grx3::LEU2 grx4::KanMX4</i>	(3)
<i>GalDRE2Δcrt1</i>	BY4741, <i>dre2::HIS3-P_{GAL1}-DRE2 crt1::KanMX4</i>	This study
SPY122	BY4741, <i>cth1::KanMX4 cth2::HIS3MX4</i>	(4)
AXY1171	BY4741, <i>dre2::HIS3-P_{GAL1}-DRE2</i>	This study
AXY1195	BY4742, <i>dre2::HIS3-P_{GAL1}-DRE2 pmr1::KanMX4</i>	This study
AXY1493	BY4741, <i>tah18::KanMX4 pMH1712</i> (pRS415- P _{TAH18} -MYC3-TAH18)	This study
AXY1494	BY4741, <i>tah18::KanMX4 pMH1713</i> (pRS415- P _{TAH18} -MYC3-tah18-5H8)	This study
AXY1495	BY4741, <i>tah18::KanMX4 pMH1714</i> (pRS415- P _{TAH18} -MYC3-tah18-5I5)	This study
AXY1542	BY4741, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4, tah18::KanMX4 pMH1712</i> (pRS415- P _{TAH18} -MYC3-TAH18)	This study
AXY1543	BY4741, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4, tah18::KanMX4 pMH1713</i> (pRS415- P _{TAH18} -MYC3-tah18-5H8)	This study
AXY1544	BY4741, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4, tah18::KanMX4 pMH1714</i> (pRS415- P _{TAH18} -MYC3-tah18-5I5)	This study
AXY1664	BY4741, <i>tah18::KanMX4::MYC3-TAH18-LEU2</i>	This study
AXY1668	BY4741, <i>tah18::KanMX4::MYC3-tah18-5H8-LEU2</i>	This study
AXY1696	BY4741, <i>tah18::KanMX4::MYC3-tah18-5I5-LEU2</i>	This study
AXY1860	BY4742, <i>dre2::HIS3-P_{GAL1}-DRE2 cth1::KanMX4 cth2::HIS3MX4</i>	This study
AXY2023	BY4741, <i>dre2::HIS3-P_{GAL1}-DRE2 aft2::KanMX4</i>	This study
AXY2026	BY4741, <i>dre2::HIS3-P_{GAL1}-DRE2 dun1::KanMX4</i>	This study
AXY2033	BY4741, <i>dre2::HIS3-P_{GAL1}-DRE2 aft1::KanMX4</i>	This study
AXY1767	BY4741, <i>dre2::KanMX4, pMH1751</i> (pRS415-P _{DRE2} -MYC3-DRE2)	This study
Y300	<i>MATa can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	(5)
AXY1233	Y300, <i>tah18-5H8</i>	This study
AXY1235	Y300, <i>tah18-5I5</i>	This study
AXY1282	Y300, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4</i>	This study
AXY1313	Y300, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4 tah18-5H8</i>	This study
AXY1317	Y300, <i>rnr4::HIS3MX6-P_{GAL1}-RNR4 tah18-5I5</i>	This study
AXY1914	Y300, <i>HIS3-P_{GAL1}-RNR4 crt1::LEU2</i>	This study
AXY1916	Y300, <i>HIS3-P_{GAL1}-RNR4 crt1::LEU2 tah18-5H8</i>	This study
AXY1918	Y300, <i>HIS3-P_{GAL1}-RNR4 crt1::LEU2 tah18-5I5</i>	This study
MHY340	Y300, <i>rnr2::KanMX6-P_{RNR2}-MYC3-RNR2</i>	This study

- Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
- Zhang Y, et al. (2011) Investigation of in vivo diferric tyrosyl radical formation in *Saccharomyces cerevisiae* Rnr2 protein: Requirement of Rnr4 and contribution of Grx3/4 AND Dre2 proteins. *J Biol Chem* 286(48):41499–41509.
- Mühlenhoff U, et al. (2010) Cytosolic monothiol glutaredoxins function in intracellular iron sensing and trafficking via their bound iron-sulfur cluster. *Cell Metab* 12(4):373–385.
- Puig S, Askeland E, Thiele DJ (2005) Coordinated remodeling of cellular metabolism during iron deficiency through targeted mRNA degradation. *Cell* 120(1):99–110.
- Allen JB, Zhou Z, Siede W, Friedberg EC, Elledge SJ (1994) The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. *Genes Dev* 8(20):2401–2415.

Table S2. Plasmids used in this study

Plasmid	Description	Source
pMH1763	pRS415-P _{DIF1} -MYC3-DIF1	This study
pMH1712	pRS415-P _{TAH18} -MYC3-TAH18	This study
pMH1713	pRS415-P _{TAH18} -MYC3-tah18-5H8	This study
pMH1714	pRS415-P _{TAH18} -MYC3-tah18-5I5	This study
pMH1751	pRS415-P _{DRE2} -MYC3-DRE2	This study

