Supplemental information

Redox-dependent Regulation of Gluconeogenesis by a Novel Mechanism Mediated by a Peroxidatic-Cysteine of Peroxiredoxin

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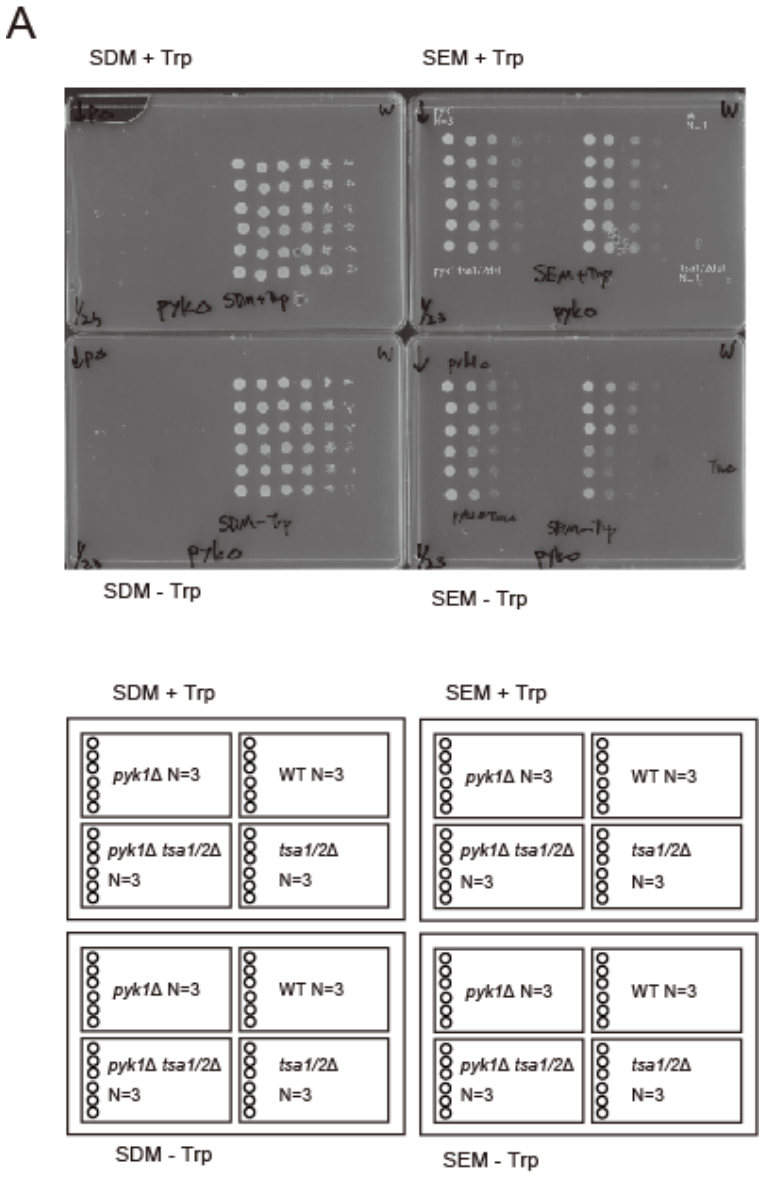

Figure supplement 1 (Related to *Figures 1D, 1E* and *3E*): Loss of Tsa1/2 leads to growth suppression in Trp- or Phe-free ethanol medium in the presence of Pyk1.
A. Original data (N = 3) for Figures 1D (upper panel) and 3E. **B**. Original data (N = 3) for Figure 1 D (lower panel). *PYK1*-disruption mutant leads to a growth defect on glucose medium[1].

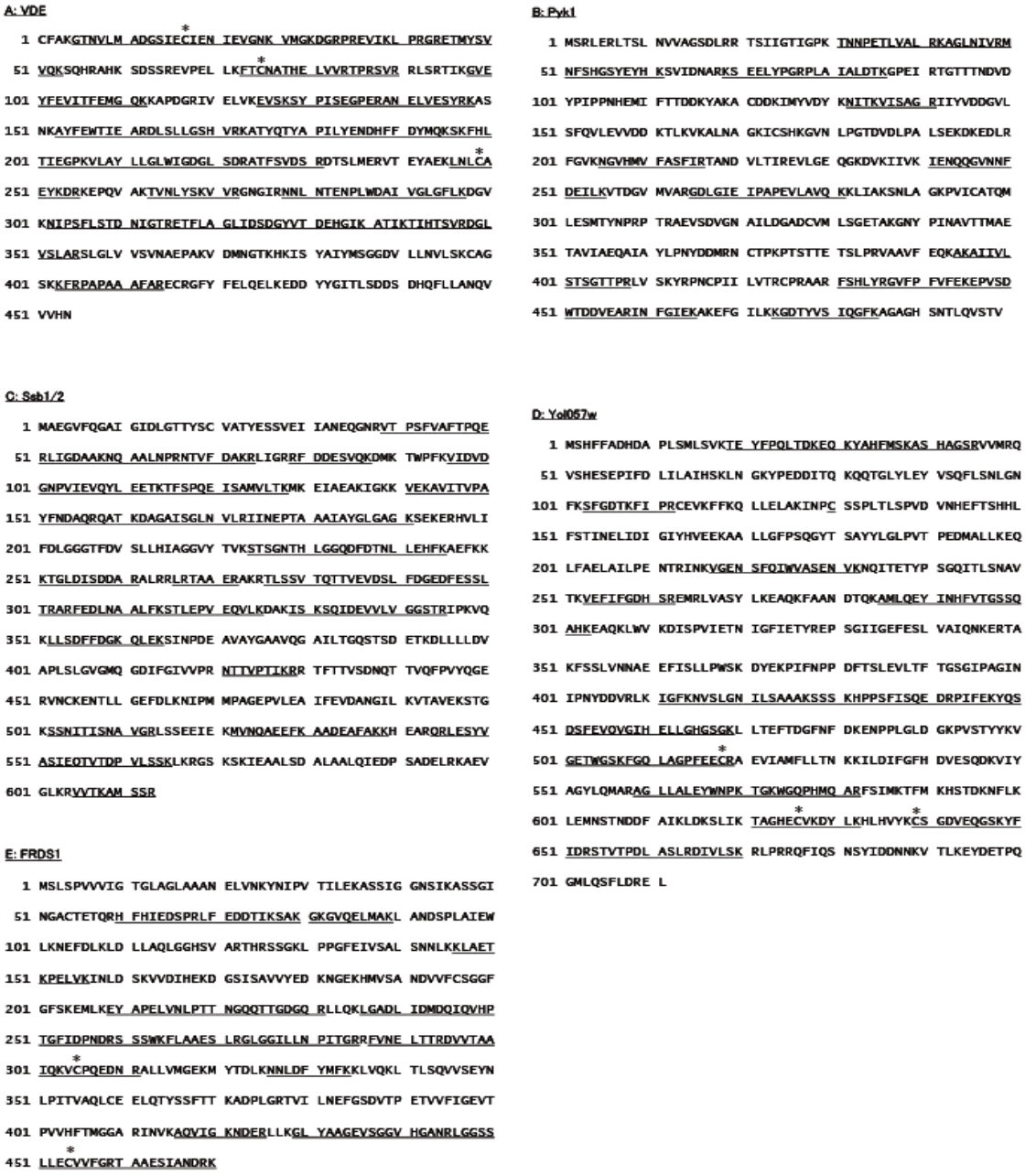

Figure supplement 2 (Related to *Figure 3C and 3D*): Isolation and identification of Tsa1 binding proteins.

Amino acid sequences for corresponding spots a-e in Figure 3C represented as A to E, respectively. Underlined regions are peptide regions that were identified in tryptic digests by peptide mass fingerprinting. Cys residues in the identified peptide are indicated as '*'. None of the Cys residues were modified by NEM, although these Cys residues were carboxymethylated by treatment during trypsinization.

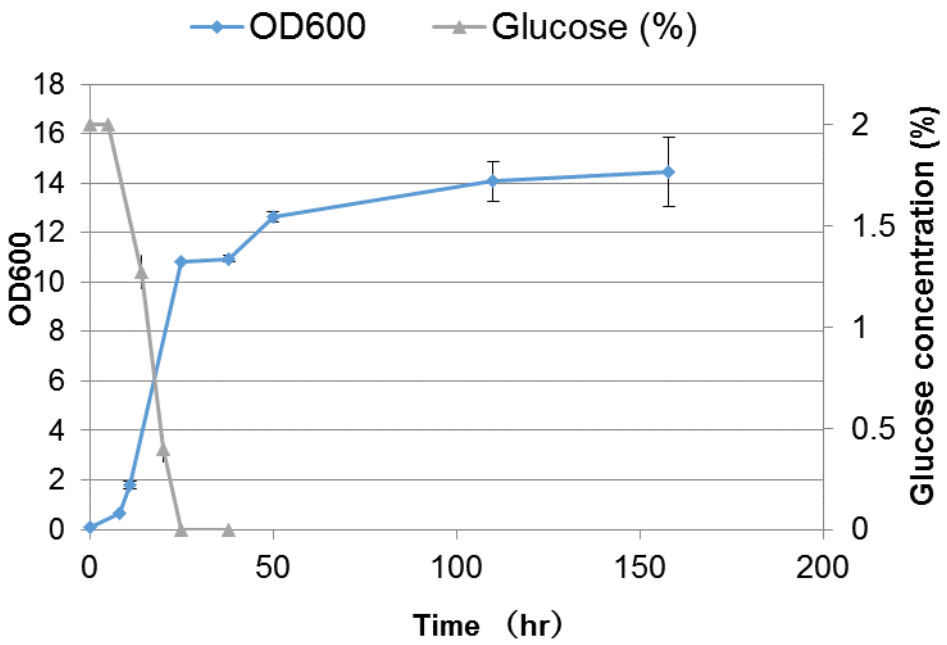

Figure supplement 3 (Related to *Figure 5B*): Wild type yeast growth and consumption of glucose in glucose medium

The growth curve and glucose consumption of wild type yeast ($PYK1^{WT}$) in SD medium. Glucose consumption was evaluated as described previously[2].

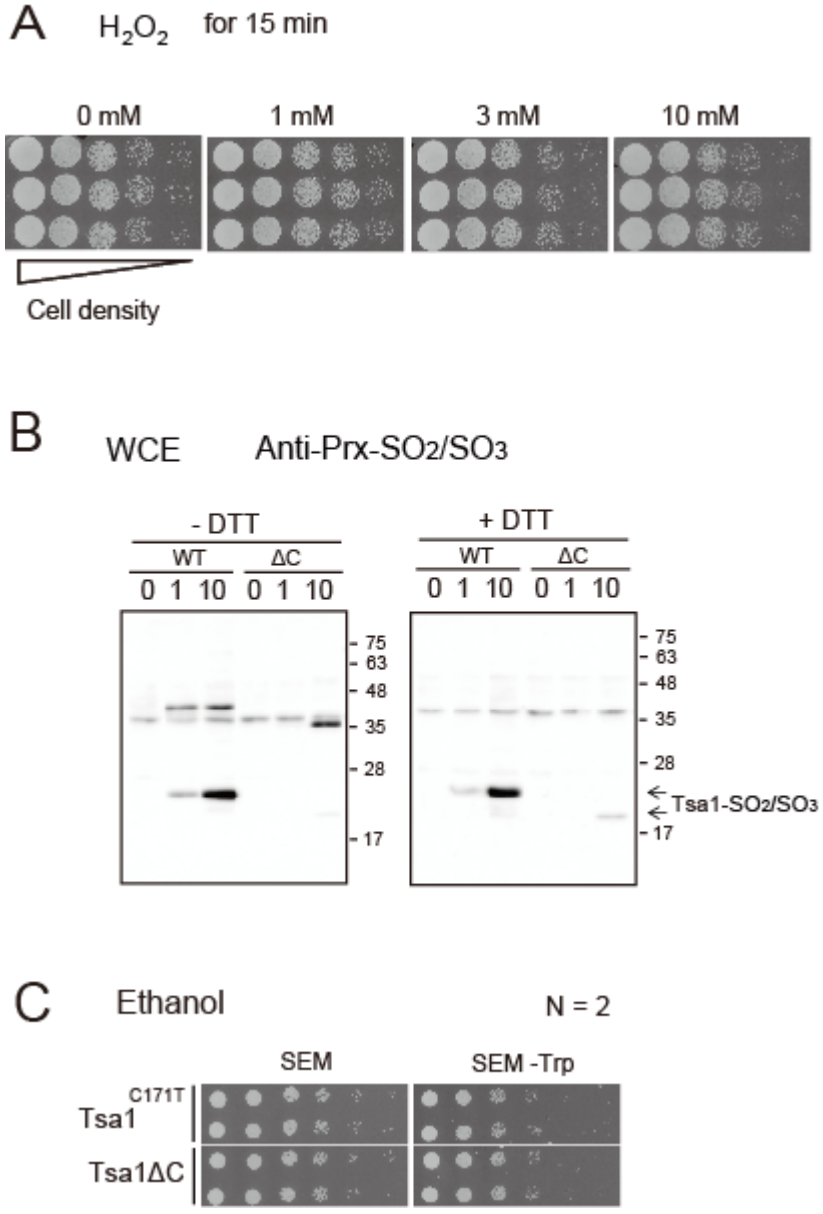

Figure supplement 4 (Related to *Figure 7*):Characterization of the hyper oxidation resistant mutant Tsa1ΔC.

A. Resistance of Yeast cells to H_2O_2. Wild type cells ($PYK1^{WT}$, N = 3) were cultured for 3 days. The cells were diluted to OD_{600} = 1 and treated with 1 mM or 10 mM or 3 mM H_2O_2 for 15 min. Then, a series of one-fifth dilutions of the cells were spotted on YPAD agar (1% Bacto peptone, 0.5% Bacto yeast extract, 2% glucose, 40 μg/ml adenine hemisulfate, 2% agar). The plates were incubated at 30°C. **B.** Whole cell Lysates described in Figure 7C were immunoblotted by anti-Prx-SO_2/SO_3 antibody. The positions Tsa1-SO_2/SO_3 and Tsa1ΔC-SO_2/SO_3 are indicated by arrows on the right side of the panels. **C**. Spot assay for tsa1/2Δ cells carrying plasmids $Tsa1^{C171T}$ and Tsa1Δ on SEM or SEM –Trp (N = 2). For plasmid selection, Leu was also omitted from the medium.

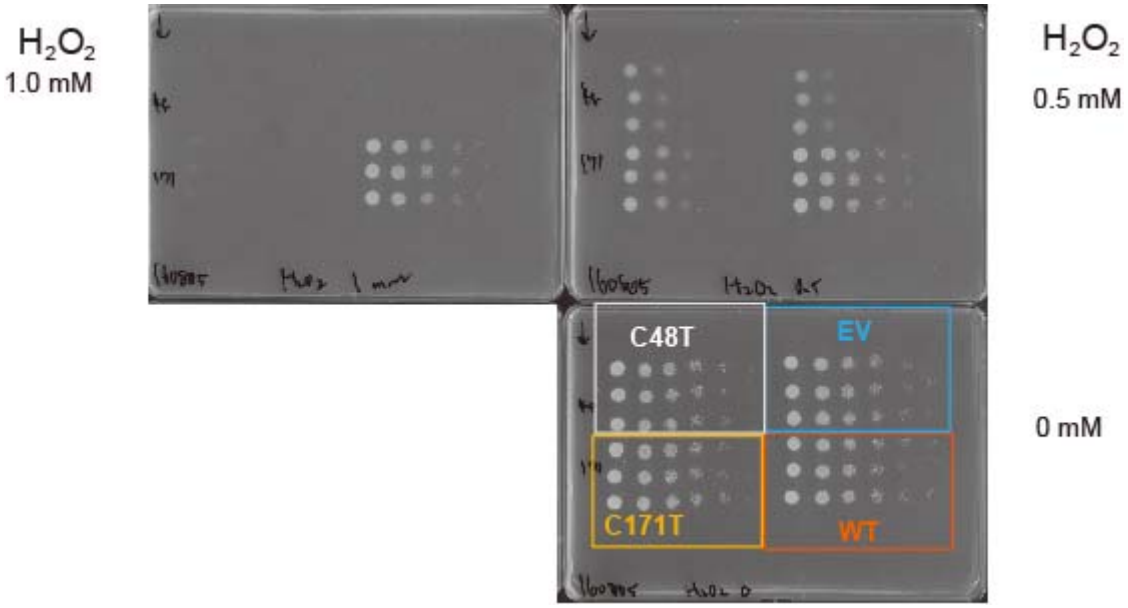

Figure supplement 5 (Related to *Figure 8*): Original image of the spot assay in Figure 8B (N = 3).

Table supplement 1 (Related to *Discussion*)

FBP (pmol / 10 OD_{600})

	Average (n = 3)	Standard error of the mean
Exponential (Glucose medium)	7626	667
Early stationary (Glucose medium)	336	17
Exponential (Ethanol medium)	425	5

FBP concentration in exponential phase cells and early stationary phase cells in SDM (glucose medium), and exponential phase cells (adapted from ***Figure 2B***) in SEM –Trp (ethanol medium).

Table supplement 2 – *Saccharomyces cerevisiae* strains used in this study (related to *Materials and Methods*)

BY4742	*MATα his3Δ1 lys2Δ0 ura3Δ0*	Euroscarf
tsa1Δ	*tsa1*::kanMX (from BY4742)	Tachibana et al. 2009
tsa1/2Δ	*tsa1*::hphMX *tsa2*::kanMX (from tsa1Δ)	Watanabe et al. 2014
pyk1Δ	*pyk1*::kanMX (from BY4742)	this study
PYKl pro::URA3	*PYK1*::*URA3* (from BY4742)	this study
PYKl pro::URA3 tsa1/2Δ	*PYK1*::*URA3* (from tsa1/2Δ)	this study
$PYK1^{WT}$	$PYK1^{WT}$ (from PYKl pro::URA3)	this study
$PYK1^{CA}$	$PYK1^{CA}$ (from PYKl pro::URA3)	this study
$PYK1^{WT}$ tsa1/2Δ	$PYK1^{WT}$ (from PYKl pro::URA3Δ tsa1/2Δ)	this study
tsa1/2Δ URA3	*tsa1*::hphMX *tsa2*::Ca*URA3* (from tsa1Δ)	Watanabe et al. 2014
pyk1Δ tsa1/2Δ	*pyk1*:: kanMX *tsa1*::hphMX *tsa2*::Ca*URA3* (from tsa1/2Δ URA3)	this study
pyk1Δ	*pyk1*:: kanMX (from BY4742)	this study

Supplementary References

1 Sprague, G. F., Jr. Isolation and characterization of a Saccharomyces cerevisiae mutant deficient in pyruvate kinase activity. *J Bacteriol* **130**, 232-241 (1977).

2 Watanabe, T., Irokawa, H., Ogasawara, A., Iwai, K. & Kuge, S. Requirement of peroxiredoxin on the stationary phase of yeast cell growth. *J Toxicol Sci* **39**, 51-58, doi:DN/JST.JSTAGE/jts/39.51 [pii] (2014).