

Supplemental information

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

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A: YDE

1 CFAKGTNVL^{*}M ADGSIECIEN IEVGNK VMGKGRPREVIKL PRGRETMYSV
51 VQKSQHRAHK SSSSREPEL LKFTCNATHE LVVRTPSRVR RLSRTIKGVE
101 YFEVITFEMG QKAPDGRIV ELVKEVSKSY PISEGPERAN ELVESYRKAS
151 NKAYFENTIE ARDLLLGGSH VRKATYQTYA PilyendHFF DYMOKSKFHL
201 TIEGPKVLAY LGLNIGDGL SDRATFSVDS RDTSLMERVT EYAEKLNLC^{*}A
251 EYKDRKEPQV AKTVNLYSKV VRNGIRNNL NTE^{*}NPLMDAI VGLGFLKDGV
301 KNIPSEFLSD NIGTR^{*}REFLA GLIDSDGYVT DEHGK ATIKTIHTSVRDGL
351 VSLARSLGLV VSVNAEPAKV DMNGTKHKIS YAIYMSGGDV LLNVLKSCAG
401 SKKFRPAPAA AFARECRGFY FELQELKEDD YYGITLSDDS DHQFLLANQV
451 VVHN

C: Seb1/2

1 MAEGVFQGA^{*}I GIDLGTYSY VATYESSVEI IANEQGNRYT PSFVAFTPQE
51 RLIGDAAKNQ AALNPRNTVF DAKRLIGRRF DDES^{*}VQKDMK TWPFKVIDVD
101 GNPVI^{*}EQYL EETKTFSPQE ISAMVLT^{*}KMK EIAEAKIGKK VEKAVITVPA
151 YFNDAQRQAT KDAGATSGLN VLRTINEPTA AATAYGLGAG KSEKERHVL I
201 FDLGGGTDFV SLLHIAGGVY TVKSTSGNTH LGGQDFDTNL LEHFKA^{*}EFKK
251 KTGLDISDDA RALRRRLTAA ERAKRTLSSV TQTTVEVDSL FDGEDFESSL
301 TRARFEDLNA ALFKSTLEPV EQVLKDAKIS K^{*}QIDEVVLV GGSTRIPK^{*}VQ
351 KLLSDFFDGK QLEKSINPDE AVAYGAAVQG AILTQQSTSD ETKDLLLLDV
401 APLSLGVGMQ GDIFGIVVPR NTVPTIKRR TFTTVSDNQT TVQFPVYQGE
451 RVNCKENTLL GEFDLKNIPM MPAGEPVLEA IFEVDANGIL KVTAVEKSTG
501 KSSNITISNA VGRLSSEEIE KMVNOAEEFK AADEFAK^{*}KH EARQRLESY^{*}Y
551 ASIEQVYDTP VLSSKLRGS KSKIEAALSD ALAALQIEDP SADELKAEV
601 GLKRVYTKAM SSR

E: FRDS1

1 MSLSPVVVIG TGLAGLAAAN ELVNKYNIPV TILEKASSIG GNSIKASSGI
51 NGA^{*}CTETQRH FHIEDSPRLF EDDTIKSAK GKG^{*}VOELMAKL ANDSP^{*}LATEW
101 LKNEFDLKL D LLAQLGGHSV ARTHRSSGKL PPGFEIVSAL SNNLKL^{*}LAET
151 KPELVKINLD SKVVDIHEKD GSISAVVYED KNGEKHMVSA NDV^{*}VFCGGF
201 GFSKEMKEY APELVNLP^{*}TT NGQQTGDGQ RLLQKLGADL IDMDQIQVHP
251 TGFIDPNDRS SSWKFLA^{*}ES LRGLGGILLN PITGRREFVNE L^{*}TTRDVVTAA
301 IQKVC^{*}PQEDN RALLVMGEKM YTDLKNLDF YMFKKLVQKL T^{*}LSQVVSEYN
351 LPITVAQLCE ELQTYSSFTT KADPLGR^{*}TVI LNEFGSDVTP ETVVFIGEVT
401 PVVHFTMGGA RINVK^{*}QVIG KNDERLLKGL YAAGEVSGGV HGANRLGGSS
451 LLEC^{*}VVFGRT AAESIANDRK

B: Pyk1

1 MSRLERLTSL NVVAGSDLR^{*}R TSIIGTIGPK TNNPETLVAL RKAGLNIVRM
51 NFSHGSYEYH KSVIDNARKS EELYPGRPLA IALDTKGPEI RTGTTNDVD
101 YPIPPNHEMI FTDDKYAKA CDDKIMYVDY KNITKVISAG RIIYVDDGVL
151 SFQVLEVVD D KTLKVKALNA GKICSHKGVN LPGTDVDLPA LSEKDEDLR
201 FGVKN^{*}GVHMV FASFIRTAND VLTIREVLGE QGKDVKIIVK IENQGVN^{*}NF
251 DELK^{*}VTDGV MVAR^{*}DGLGIE IPAPEVLAVQ KKI^{*}AKSNLA GKPVICATQM
301 LESMTYNPRP TRAEVSDVGN AILDGADCVN LSGETAKGNY PINAVTTMAE
351 TAVIAEQ^{*}IA YLPNYDDMRN CTPKPTSTTE TSLPRVA^{*}AV EQKAK^{*}AITVL
401 SISGTT^{*}PRLV SKYRPNCP^{*}II LVTRCPRAAR FSHLYRGVFP FVFEKEPVSD
451 WTDDVEARIN FGIEKAKEFG ILKKGDTYVS IQGFKAGAGH SNTLQVSTV

D: Yel057w

1 MSHFFADHDA PLSMLSVKTE YFPQLT^{*}DKEQ KYAHFMSKAS HAGSRVVMRQ
51 VSHSEPIFD LILAIHSKLN GKYPEDDITQ KQQTGLYLEY VSQFLSNLGN
101 FKSFGDTKFI PRCEV^{*}KFFKQ LLELAKINPC SSPLT^{*}LSPVD VNHEFTSHHL
151 FSTINELIDI GIYHVEEKAA LLGFP^{*}SQGYT SAYYGLPV^{*}T PEDMALLKEQ
201 LFAELAILPE NTRINKVGEN SFOI^{*}WASEN VKNQITETYP SGQITLSNAV
251 TKVEFIFGDH SREMLVASY LKEAQKFAAN DTQKAM^{*}LQ^{*}EY INHEVTGSSQ
301 AHKEAQLWV KDISPVIETN IGF^{*}IETYREP SGI^{*}GEFESL VAIQNKERTA
351 KFS^{*}SLVNAE EFISLLPWSK DYKPIFNPP DFTSLEVLTF TGSGIPAGIN
401 IPNYDDVRLK IGFKNVSLGN ILSAAKSSS KHPPSFI^{*}SQE DRPIFEKYQS
451 DSFEVOYGIH ELLGHGSGKL L^{*}TEFTDGNF DKENPPLGLD GKPVSTY^{*}YK
501 GETWGSKEGQ LAGPFE^{*}ECRA EVIAMFL^{*}LTN KKILDIFGFH DVESQDKVIY
551 AGYLQMARAG LLALEY^{*}WNP^{*}K TGK^{*}WQPHMQ ARFSIMK^{*}TFM KHSTDKNFLK
601 LEMNSTNDDF AIKLDKSLIK TAGHECVKDY LKHLHVYKCS GDVEQGSKYF
651 IDRSTVTPDL ASLRD^{*}IVLSK RLP^{*}RRQFIQS NSYIDONNKV TLKEYDET^{*}PQ
701 GMLQSF^{*}LDRE L

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.

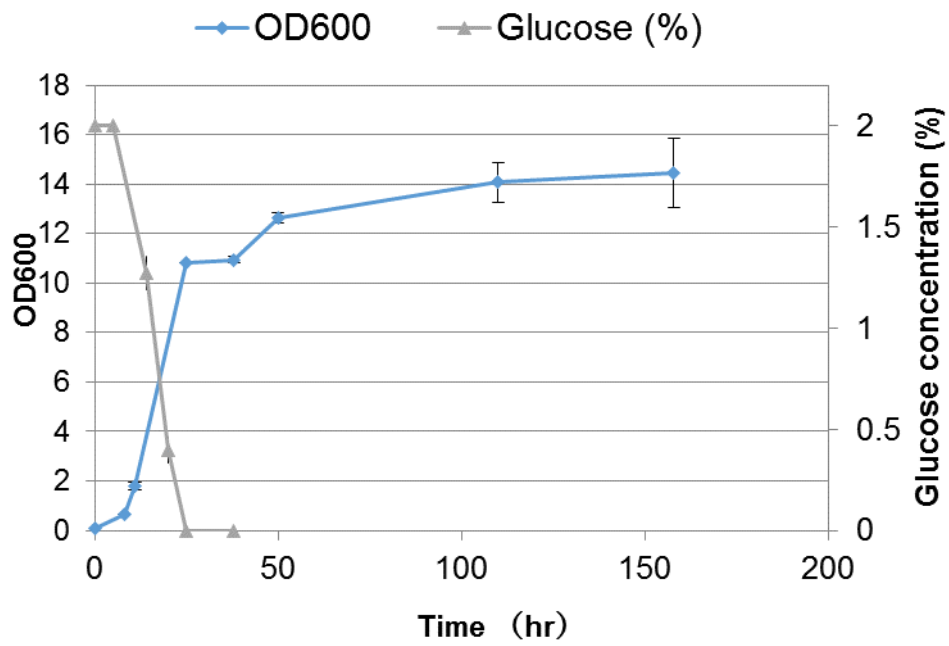


Figure supplement 3 (Related to *Figure 5B*): Wild type yeast growth and consumption of glucose in glucose medium
 The growth curve and glucose consumption of wild type yeast (PYK1^{WT}) in SD medium. Glucose consumption was evaluated as described previously².

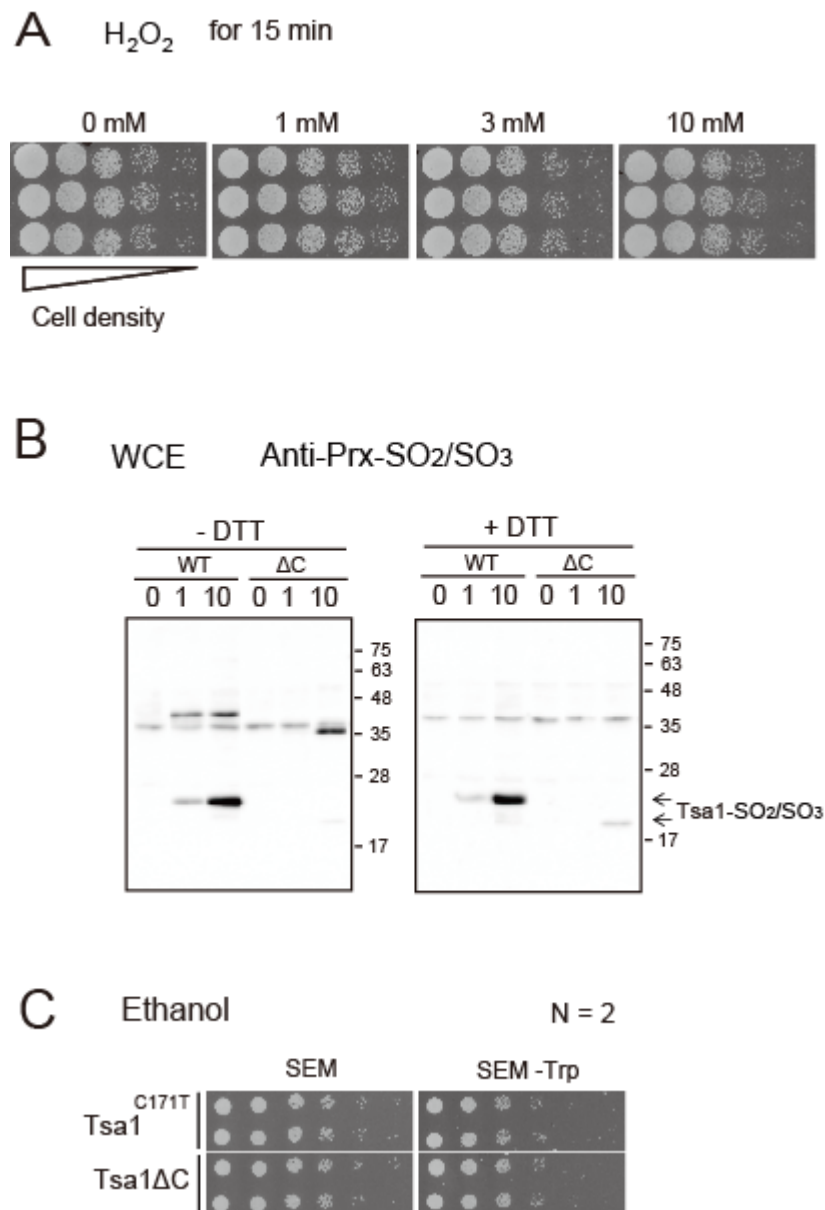


Figure supplement 4 (Related to Figure 7): Characterization of the hyper oxidation resistant mutant Tsa1ΔC.

A. Resistance of Yeast cells to H₂O₂. Wild type cells (PYK1^{WT}, N = 3) were cultured for 3 days. The cells were diluted to OD₆₀₀ = 1 and treated with 1 mM or 10 mM or 3 mM H₂O₂ 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-SO₂/SO₃ antibody. The positions Tsa1-SO₂/SO₃ and Tsa1ΔC-SO₂/SO₃ 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 OD₆₀₀)

	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	<i>MATa his3Δ1 lys2Δ0 ura3Δ0</i>	Euroscarf
<i>tsa1Δ</i>	<i>tsa1::kanMX</i> (from BY4742)	Tachibana et al. 2009
<i>tsa1/2Δ</i>	<i>tsa1::hphMX tsa2::kanMX</i> (from <i>tsa1Δ</i>)	Watanabe et al. 2014
<i>pyk1Δ</i>	<i>pyk1::kanMX</i> (from BY4742)	this study
PYK1 pro::URA3	<i>PYK1::URA3</i> (from BY4742)	this study
PYK1 pro::URA3 <i>tsa1/2Δ</i>	<i>PYK1::URA3</i> (from <i>tsa1/2Δ</i>)	this study
PYK1 ^{WT}	<i>PYK1^{WT}</i> (from PYK1 pro::URA3)	this study
PYK1 ^{CA}	<i>PYK1^{CA}</i> (from PYK1 pro::URA3)	this study
PYK1 ^{WT} <i>tsa1/2Δ</i>	<i>PYK1^{WT}</i> (from PYK1 pro::URA3Δ <i>tsa1/2Δ</i>)	this study
<i>tsa1/2Δ</i> URA3	<i>tsa1::hphMX tsa2::CaURA3</i> (from <i>tsa1Δ</i>)	Watanabe et al. 2014
<i>pyk1Δ tsa1/2Δ</i>	<i>pyk1::kanMX tsa1::hphMX tsa2::CaURA3</i> (from <i>tsa1/2Δ</i> URA3)	this study
<i>pyk1Δ</i>	<i>pyk1::kanMX</i> (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).