**Supporting Information.**

**Supplementary Methods.**

**Generation and Characterization of *tsa1Δ tsa2Δ* strain.** To create a targeting construct for disrupting the *TSA2* locus, 500 bp upstream and downstream of the *TSA2* coding sequence (5'-UTR and 3'-UTR, respectively) were amplified out of *S. cerevisiae* genomic DNA using the primers described in Supplementary Table 1 with standard PCR conditions. The amplified products were sequentially cloned into pBluescript II vector containing a *HIS3* rescue cassette cloned into the BamHI site using the restriction sites described in the primer sequence. Correct production of the targeting construct was confirmed by restriction digestion and DNA sequencing. Wild-type (BY4741) or *tsa1Δ::kan*$^R$ strains were transformed with the targeting construct and plated to His dropout medium. PCR genotyping was performed to confirm disruption of the *TSA2* locus (Supp. 7A). The strain's phenotype was also characterized for $H_2O_2$ sensitivity (Supp. Fig. 7B).

**Supplementary Table 1. Primers Used for Cloning, Site-Directed Mutagenesis, and Deletion Mutant Production.**

Primers for Cloning Tsa1 into pET45b (KpnI/XhoI)

Forward: 5'-GGGGGGGTACCGTCGCTCAAGTTCAAAAGCAAG
Reverse: 5'-CCCCCCTCGAGTTATTTGTTGGCAGCTTCGAAG

Primers for Cloning FLAG-fusion proteins into p416-GPD (FLAG tag underlined)

FLAG-Trx2 (SpeI/XhoI)
Forward: 5'-GGGGACTAGTATGGATTACAAAGATGATGATGATAAAGTCACTCAATTAAAATCCGCTTC
Reverse: 5'-GGGGCTCGAGCTATACGTTGGAAGCAATAGCTTGC

FLAG-Tsa1 (SpeI/XhoI)
Forward: 5'-GGGGACTAGTATGGATTACAAAGATGATGATGATAAAGTCGCTCAAGTTCAAAAGCAAGC
Reverse: 5'-GGGGCTCGAGTTATTTGTTGGCAGCTTCGAAGTATTCC

FLAG-Trr1 (HindIII/XhoI)
Forward: 5'-GGGGAAGCTTATGGATTACAAAGATGATGATGATAAAGTTCACAACAAAGTTACTATC
Reverse: 5'-GGGGCTCGAGCTATTCTAGGGAAGTTAAGTATTTCTCAG

Site-Directed Mutagenesis Primers (Mutation Sites in Bold)

$C^{31}A$-Trx2
Forward: 5'-CTTTTTTGCCACATGG**GCT**GGGCCATGTAAAATGATTGC
Reverse: 5'-GCAATCATTTTACATGGCCC**AGC**CCATGTGGCAAAAAAG

$C^{34}A$-Trx2
Forward: 5'-GCCACATGGTGTGGGCCA**GCT**AAAATGATTGCACCAATG
Reverse: 5'-CATTGGTGCAATCATTTT**AGC**TGGCCCACACCATGTGGC

$C^{31,34}A$-Trx2
Forward: 5'-GCCACATGG**GCT**GGGCCA**GCT**AAAATGATTGCACCAATG
Reverse: 5'-CATTGGTGCAATCATTTT**AGC**TGGCCC**AGC**CCATGTGGC

$C^{47}A$-Tsa1
Forward: 5'-CCATTGGCCTTCACTTTCGTC**GCT**CCAACCGAAATCATTGCTTTC
Reverse: 5'-GAAAGCAATGATTTCGGTTGG**AGC**GACGAAAGTGAAGGCCAATGG

$C^{170}A$-Tsa1
Forward: 5'-AAGAACGGTACTGTCTTGCCA**GCT**AACTGGACTCCAGGTGCTGCT
Reverse: 5'-AGCAGCACCTGGAGTCCAGTT**AGC**TGGCAAGACAGTACCGTTCTT

Primers for *tsa2Δ* Targeting Construct

Tsa2 5'-UTR primers (XhoI/EcoRI)
Forward: 5'-GGGGCTCGAGGTTCTCAACGGGCTTATGCTAG
Reverse: 5'-GGGGGAATTCGAACTTCTGCTACCATGATTGG

Tsa2 3'-UTR primers (SpeI/NotI)
Forward: 5'-GGGGACTAGTGCCAATAATTAATCTTCGCACG
Reverse: 5'-GGGGGCGGCCGCGACAAGACTATGCCAATTGAG

Primers for Genotyping *tsa1Δ* and *tsa2Δ* Strains

wt *TSA1* locus
Forward: 5'-TGCGTTTAAGGTGTACGAAAACCC
Reverse: 5'-AAACCACCTTCCTTTCTTGGGA

wt *TSA2* locus
Forward: 5'-ATTATCTTAACTATATGCGCCCCTC
Reverse: 5'-CATGCCAGTAAGGAATATTCAGAGT

*tsa1Δ::kan*$^{R}$ locus
Forward: 5'-TGCGTTTAAGGTGTACGAAAACCC
Reverse: 5'-CTGCAGCGAGGAGCCGTAAT

*tsa2Δ::kan*$^{R}$ locus
Forward: 5'-ATTATCTTAACTATATGCGCCCCTC
Reverse: 5'-CTGCAGCGAGGAGCCGTAAT

*tsa2Δ::HIS3* locus
Forward: 5'-GATTAGCGACCAGCCGGAATGC
Reverse: 5'-ACTCAATGGATTCGTTAACAGTAGG

**Supplementary Table 2. Summary of Proteomic Data from FLAG-Trx2 Immunoprecipitations.**[a]

**Proteins Co-Precipitating from $Me_2SO$-Treated Cells.**

| Protein | Biological Function[b] | Predicted MW[c] | Approximate MW on Gel | Mascot Score (Expt. 1, Expt. 2)[d] | No. of Peptides Identified (Expt. 1, Expt. 2)[e] |
|---|---|---|---|---|---|
| Trx2/Trx1 | protein disulfide reductase | 11,311 | 12,000 | (282, 180) | (15, 8) |
| | | | 18,000 | (59, 70) | (3, 2) |
| | | | 24,000 | (80, 64) | (3, 3) |
| | | | 30,000 | (68, 66) | (3, 2) |
| | | | 36,000 | (38, 77) | (2, 2) |
| Gpx2 | peroxidase | 18,622 | 12,000 | (40, 22) | (1, 1) |
| Arf1 | ADP-ribosylation factor | 20,574 | 18,000 | (72, 72) | (9, 2) |
| Tef1/Tef2 | translation elongation factor | 50,400 | 48,000 | (29, 39) | (2, 3) |
| Tif1/Tif2 | translation initiation factor | 44,840 | 54,000 | (42, 59) | (3, 4) |
| Ssa1/Ssa2 | molecular chaperone | 69,786 | 72,000 | (65, 38) | (5, 3) |
| Ssb1 | molecular chaperone | 69,599 | 72,000 | (40, 32) | (5, 1) |
| Hsc82/Hsp82 | molecular chaperone | 80,850/81,356 | 78,000 | (197, 260) | (14, 12) |

**Proteins Co-Precipitating from DVSF-Treated Cells.**

| Protein | Biological Function[b] | Predicted MW[c] | Approximate MW on Gel | Mascot Score (Expt. 1, Expt. 2)[d] | No. of Peptides Identified (Expt. 1, Expt. 2)[e] |
|---|---|---|---|---|---|
| Trx2/Trx1 | protein disulfide reductase | 11,311 | 12,000 | (215, 206) | (8, 7) |
| | | | 18,000 | (110, 85) | (4, 2) |
| | | | 24,000 | (57, 108) | (2, 3) |
| | | | 30,000 | (77, 33) | (3, 1) |
| | | | 36,000 | (230, 128) | (7, 4) |
| | | | 42,000 | (230, 144) | (7, 4) |
| | | | 48,000 | (192, 116) | (7, 3) |
| | | | 54,000 | (259, 27) | (16, 1) |
| | | | 60,000 | (99, 69) | (5, 3) |
| | | | 66,000 | (94, 105) | (4, 2) |
| | | | 72,000 | (120, 65) | (4, 2) |
| | | | 78,000 | (88, 76) | (3, 2) |
| | | | 84,000 | (92, 92) | (4, 3) |
| Arf1 | ADP-ribosylation factor | 20,574 | 18,000 | (86, 66) | (3, 2) |
| Tsa1 | peroxidase | 21,690 | 24,000 | (104, 116) | (4, 3) |
| | | | 30,000 | (46, 160) | (2, 4) |
| | | | 36,000 | (188, 69) | (8, 2) |
| | | | 42,000 | (188, 138) | (8, 8) |
| | | | 48,000 | (130, 75) | (6, 2) |
| | | | 54,000 | (285, 45) | (10, 2) |
| | | | 60,000 | (250, 213) | (10, 8) |
| | | | 66,000 | (259, 142) | (11, 6) |
| | | | 72,000 | (168, 87) | (6, 4) |

| | | | | | |
|---|---|---|---|---|---|
| | | | 78,000 | (87, 98) | (5, 3) |
| Tsa2 | peroxidase | 21,715 | 42,000 | (119, 60) | (6, 5) |
| | | | 54,000 | (83, 45) | (10, 2) |
| | | | 60,000 | (57, 66) | (5, 3) |
| Tef1/Tef2 | translation elongation factor | 50,033 | 54,000 | (24, 42) | (3, 1) |
| Trr1 | thioredoxin reductase | 34,445 | 54,000 | (116, 52) | (8, 2) |
| Ssa1/Ssa2 | molecular chaperone | 69,786/69,599 | 72,000 | (125, 70) | (11, 4) |
| Ssb1 | molecular chaperone | 66,732 | 78,000 | (26, 41) | (2, 1) |
| Hsc82/Hsp82 | molecular chaperone | 80,850/81,356 | 86,000 | (121, 61) | (8, 3) |

[a]Proteins listed were isolated and identified in two replicate experiments under the treatment conditions outlined in Experimental Procedures. [b]Biological function information was obtained from www.yeastgenome.org. [c]Predicted molecular weight (MW) values were obtained from www.expasy.org. [d]Mascot database scores from the replicate experiments are provided, with higher numbers indicating the relative likelihood that a correct identification of the protein has been made. Only proteins with Mascot scores of greater than 20 were included in further analysis. [e]The number of peptides identified for each protein isolated is included from each of the replicate experiments.

**Supplementary Table 3. Relative Toxicities of DVSF, DAD, DEB, HN2 in RKO Cells.**

| Molecule | $IC_{50}$ (μM)[a] |
|---|---|
| DVSF | 34 ± 3[b] |
| DAD | 30 ± 1[c] |
| DEB | 130 ± 40 |
| HN2 | 10 ± 2 |

[a]Toxicity was measured using the WST-1 assay described. $IC_{50}$ values (the concentration at which WST-1 conversion was 50% of the control) were determined using GraphPad Prism. [b]Value is from *Chem. Res. Toxicol.* (2011) **24**, 1457-1459. [c]Value is from *Chem. Res. Toxicol.* (2011) **24**, 81-88.

**Supplementary Figure Legends.**

**Supplementary Figure 1. Representative Mass Spectra to Establish Identity of Trx2.** Two representative collision-induced dissociation (CID) spectra showing the fragmentation of peptides derived from Trx2. In this case, the Trx2 peptides migrated at the same location on the gel where a cross-linked Tsa1 species also electrophoresed (at ~36 kDa). The same peptides were isolated in the gel region where a cross-linked Trr1 was isolated (at ~54 kDa).

**Supplementary Figure 2. Representative Mass Spectra to Establish Identity of Tsa1.** Two representative CID spectra showing the fragmentation of peptides derived from Tsa1. The digested cross-linked species of Tsa1 that yielded these peptides migrated with Trx2 at ~36 kDa.

**Supplementary Figure 3. Representative Mass Spectra to Establish Identity of Trr1.** Two representative CID spectra showing the fragmentation of peptides derived from Trr1. The digested cross-linked species of Trr1 that yielded these peptides migrated with Trx2 at ~54 kDa.

**Supplementary Figure 4. Modification of Trr1-TAP by DVSF.** Yeast cultures expressing Trr1-TAP were exposed to increasing concentrations of DVSF. Protein lysates from treated cells were resolved by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies against the TAP tag (anti-protein A) or against Pgk1 (loading control). Cross-linked complexes are indicated with arrows. Results are representative of two independent experiments.

**Supplementary Figure 5. Toxicity of DEB and HN2 in *S. cerevisiae* and RKO Cells.** (A) Yeast cells in mid-log phase were exposed to $Me_2SO$ (vehicle) or varying doses of DEB or HN2

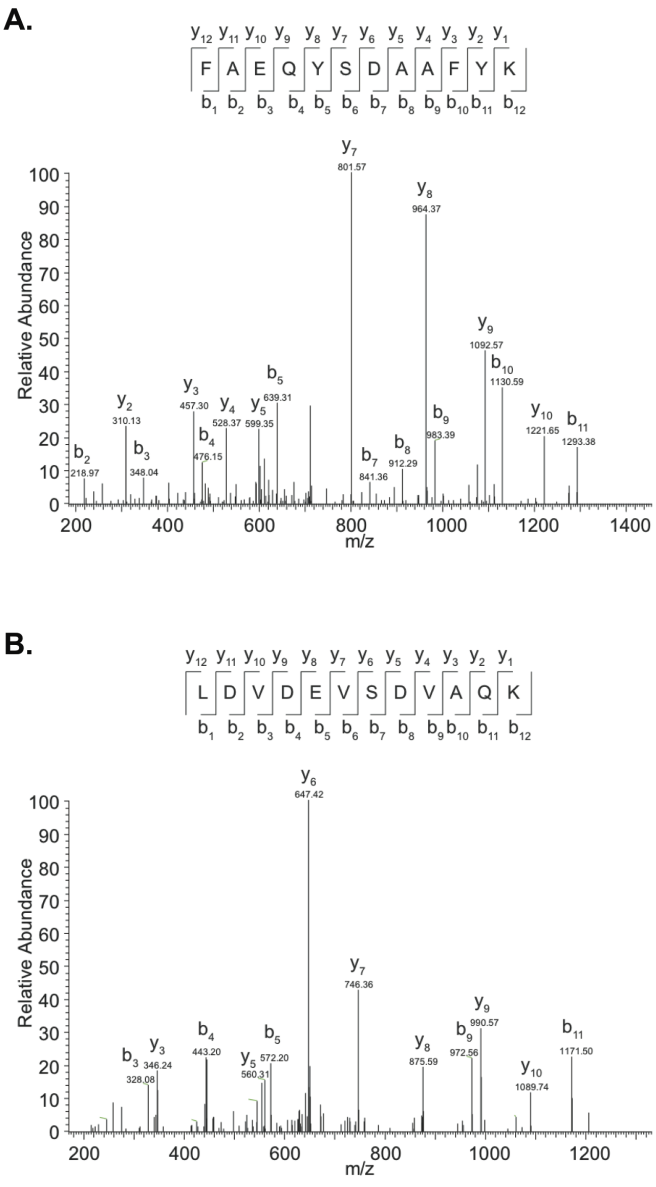
for 1 h. Cultures were diluted in a series of 10-fold dilutions, spotted onto YPD medium, and monitored for survival/growth after 48 h at 30°C. Results are representative of two independent experiments. (B) RKO cells were incubated with DEB or HN2 over a concentration range from ~320 nM to 1 mM for 24 h at 37°C. Viability was measured using the WST-1 assay, with 100% survival set at the amount of WST-1 conversion in the $Me_2SO$ (vehicle) control. Results shown are the average of two independent experiments done in quadruplicate $\pm$ SEM.

**Supplementary Figure 6. Decreased Cross-Linking of FLAG-Trx2 in Yeast Lacking Tsa1 and Tsa2 Following Exposure to Structurally Diverse Bifunctional Electrophiles.** Wild-type (BY4741) or *tsa1Δ tsa2Δ* yeast expressing FLAG-Trx2 were exposed to 300 μM DVSF for 1 h. Protein lysates from cultures were subjected to immunoblot for the FLAG tag (to detect changes in cross-linked complexes containing Trx2) or Pgk1 (loading control). An asterisk indicates a non-specific band. Results are representative of two independent experiments.

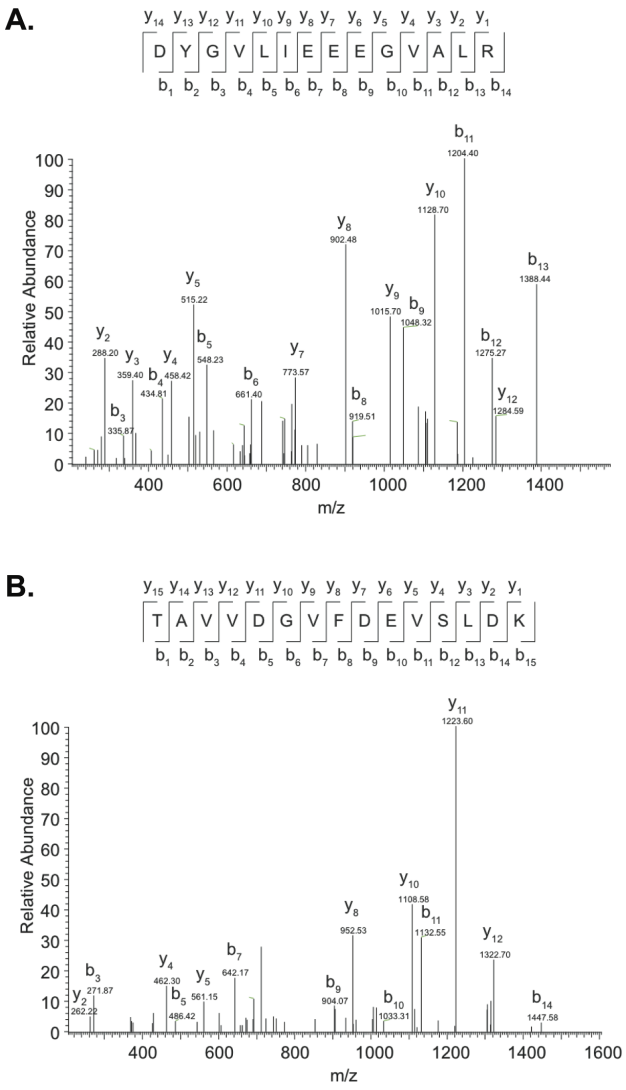
**Supplementary Figure 7. Active Site Cys Residues Mediate Cross-Linking of Recombinant Tsa1 Treated with DVSF or DAD.** Wild-type (wt) or mutant Tsa1 (10 μM) was treated with $Me_2SO$ (vehicle), DVSF, or EVSF (A) or $Me_2SO$, DAD, or DEM (B) for 24 h at 37°C as described in the Materials and Methods. Samples were resolved using SDS-PAGE and visualized with Coomassie blue staining. Results are representative of two-three independent experiments.

**Supplementary Figure 8. Genotypic and Phenotypic Characterization of the *tsa1Δ tsa2Δ* Mutant.** (A) Validation of strain by genotyping. Genomic DNA was isolated from the yeast

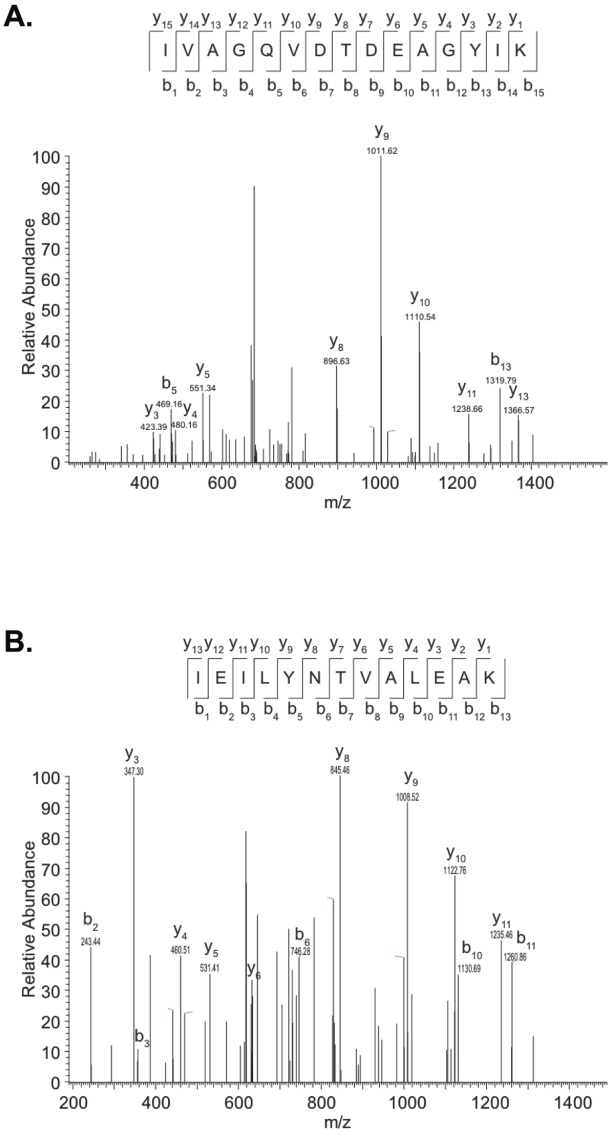
strains shown at the top of the figure. PCRs were set up with primers specific for the wild-type (wt) loci for *TSA1* and *TSA2*, a deleted *tsa1* gene (where gene disrupted with a $kan^R$ cassette), or a deleted *tsa2* gene (where gene was disrupted with either a $kan^R$ cassette or *HIS3* gene). The *tsa1Δ tsa2Δ* strain shows PCR products consistent with both genes being disrupted. (B) Phenotypic characterization of strains with hydrogen peroxide sensitivity. Serial dilutions of strains diluted to $OD_{600}$ = 0.5 from stationary phase cultures were plated onto YPD plates containing $Me_2SO$ (vehicle) or 3 mM $H_2O_2$ and grown for 48 h at 37°C. As expected, the *tsa1Δ* strain and the *tsa1Δ tsa2Δ* strain showed increased sensitivity to $H_2O_2$ when compared with the wt control. Results are representative of three independent experiments.



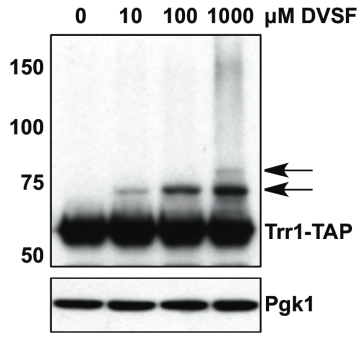

**Supplementary Figure 1**



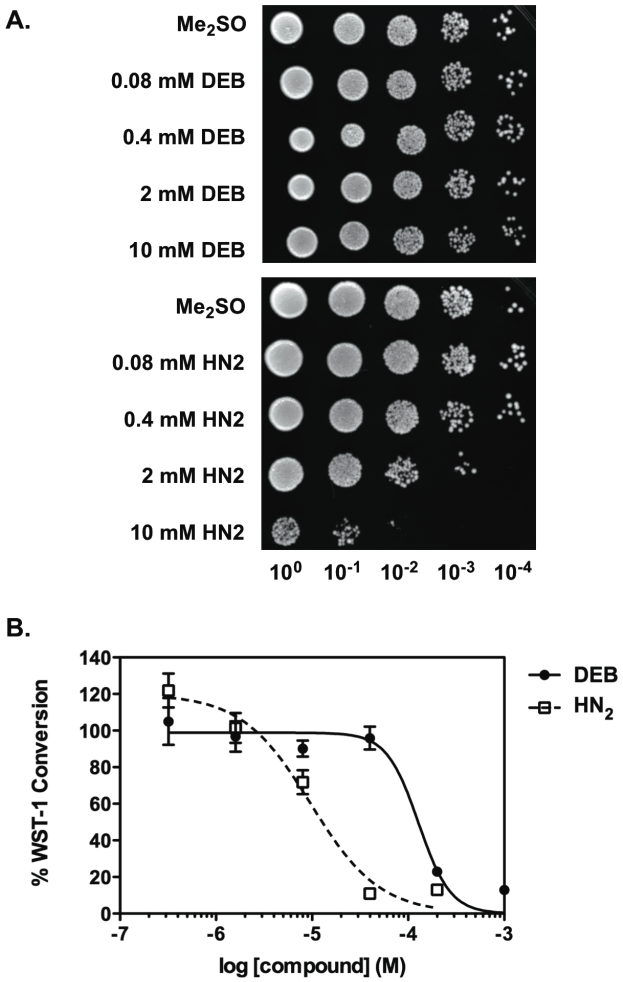

**Supplementary Figure 2**



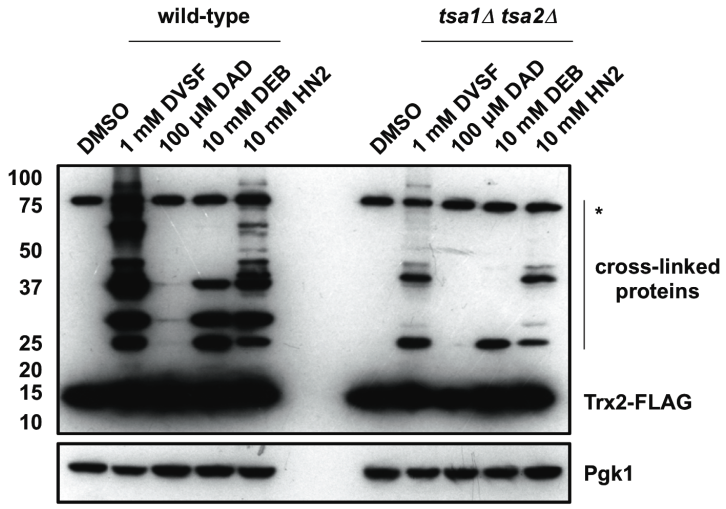

**Supplementary Figure 3**



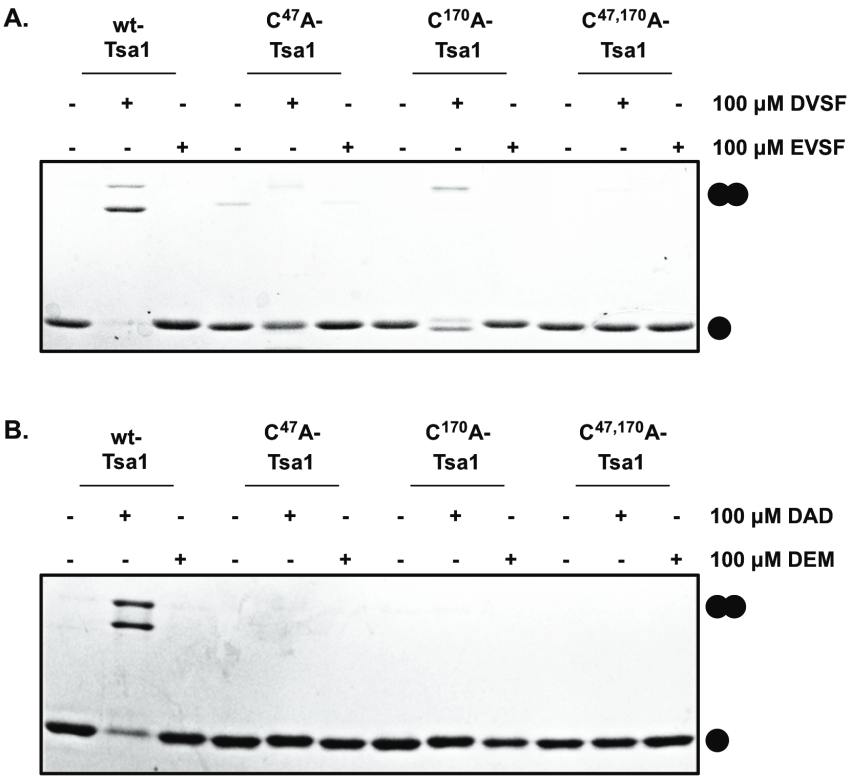

**Supplementary Figure 4**



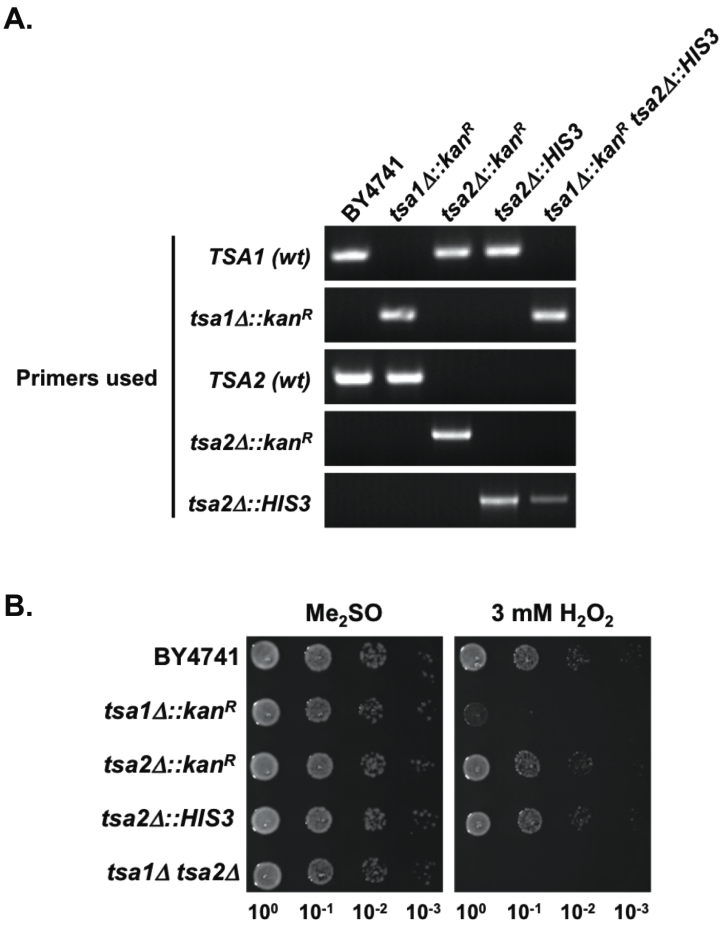

**Supplementary Figure 5**




**Supplementary Figure 6**




**Supplementary Figure 7**




**Supplementary Figure 8**