SUPPLEMENTARY INFORMATION

Cloning and purification. The coding region of *GTT2* gene was amplified by PCR using *Saccharomyces cerevisiae* S288C genomic DNA as template, and ligated into the pET29 expression vector (Novagen). pET29-Gtt2ΔN18 was used to generate mutants by PCR-based site-directed mutagenesis. Seven mutants were constructed, T24Y, G27A, G27S, G27C, G27F, S129A and H133A. Plasmids were transformed into *Escherichia coli* strain BL21 (DE3) (Novagen) and induced by isopropyl β-D-1-Thiogalactopyranoside (IPTG) for overexpression. Cells were harvested after 4 hr at 37°C and sonicated. After centrifugation, target proteins in supernatant were purified by a HiTrap nickel-chelating column and a HiLoad 16/60 Superdex 75 column (GE Healthcare) equilibrated with 20 mM Tris, pH 7.0, 200 mM NaCl (for crystallization), or 0.1M sodium acetate, pH 5.0 (for enzymatic assays). Protein purity was assessed by SDS-PAGE.

Crystallization. Each crystallization drop contained 1 μl protein sample at 7 mg/ml with 1 μl reservoir solution of 1.8 M $(NH_4)_2SO_4$, 20 mM DTT. Apo-form crystals grew to approximately 0.4 × 0.4 × 0.3 mm^3 in about 3 days. Crystals of GTS-bound form were obtained after one week in drops containing 8 mg/ml protein, 100 mM Tris, pH 8.5, 1.8 M $(NH_4)_2SO_4$, 20 mM DTT and 3 mM GTS. Crystals of GSH-bound form were obtained after two days in drops containing 10 mg/ml protein, 100 mM Tris, pH 8.5, 2.0 M $(NH_4)_2SO_4$, 20 mM DTT and 5 mM GSH.

Spectroscopic evidence for GSH ionization. To demonstrate the ionization of GSH upon binding to Gtt2, we measured the differential spectrum of the Gtt2-GSH complex in the presence of a non-saturating concentration of GSH (2 mM) in 0.1M PBS pH 6.5 at 25ºC (Caccuri et al, 2001). An absorption peak at 240 nm, characteristic of thiolate anion, was observed (Supplementary Figure S1A). Based on the peak value and an extinction coefficient of $\varepsilon_{240\ nm}$ = 5000 $M^{-1}cm^{-1}$, we predicted that at least 37% of the active sites bound deprotonated GSH under these conditions. This percentage may have been higher, because the active sites were apparently far from saturation at 2 mM GSH concentration, since the K_m^{GSH} for Gtt2 is 5 mM (Choi et al, 1998). The thiolate absorption peaks at different pH were obtained in 0.1 M sodium acetate buffer from pH 4.5 to pH 5.5, or in 0.1 M PBS buffer from pH 6.0 to pH 7.5. As calculated with Equation 1, the pH dependence of the thiolate absorption peaks gave a pK_a value for bound GSH of about 6.08 ± 0.15 (Supplementary Figure S1B). Spectroscopic measurement provided the evidence that Gtt2 could lower the pKa of GSH, similar to the classic GSTs.

$$Y=Y_{lim}/(1+10^{(pKa-pH)}) \qquad \text{(Equation 1)}$$

Circular dichroism assays. Circular dichroism (CD) spectra were collected with a Jasco J-810 system (Jasco Instruments, Tokyo, Japan). Wild-type Gtt2ΔN18 and seven mutants were studied in native conditions, with a protein concentration of 0.1 mg/mL in 10 mM sodium acetate (pH 5.0) recorded from 190 to 250 nm. CD curves were fitted using the Jasco J-810 accessory secondary structure analysis program to

estimate protein secondary structures (Yang et al, 1986). The results showed that the mutations did not introduce drastic changes to the protein structures (Supplementary Figure S2).

REFERENCES

Caccuri AM, Antonini G, Board PG, Flanagan J, Parker MW, Paolesse R, Turella P, Federici G, Lo Bello M, Ricci G (2001) Human glutathione transferase T2-2 discloses some evolutionary strategies for optimization of substrate binding to the active site of glutathione transferases. *J Biol Chem* **276**(8)**:** 5427-5431

Choi JH, Lou W, Vancura A (1998) A novel membrane-bound glutathione S-transferase functions in the stationary phase of the yeast Saccharomyces cerevisiae. *J Biol Chem* **273**(45)**:** 29915-29922

Yang JT, Wu CS, Martinez HM (1986) Calculation of protein conformation from circular dichroism. *Methods Enzymol* **130:** 208-269

FIGURE LEGENDS

Figure S1. Spectroscopic evidence for GSH ionization. **A)** Differential spectrum of the protein-bound GSH thiolate anion at pH 6.5. The peak value is approximately 240 nm. **B)** The [GS^-]/[active site] value was calculated from the 240 nm absorption

bands of differential spectrum obtained at different pH values. The solid line shows the best fit of the data to Equation 1.

Figure S2. Circular dichroism curves of the eight protein samples. The curves demonstrate that the mutations did not introduce drastic conformation changes.

Figure S3. Ramachandran plot of apo-form Gtt2 for a general case and with glycine (Generated by *MolProbity*). The red arrow shows Gly27, in a region allowable for a non-glycine or glycine residue, so mutation from Gly to Ala, Ser, Cys or Phe might not cause drastic structural changes to the protein.

Figure S1A&S1B

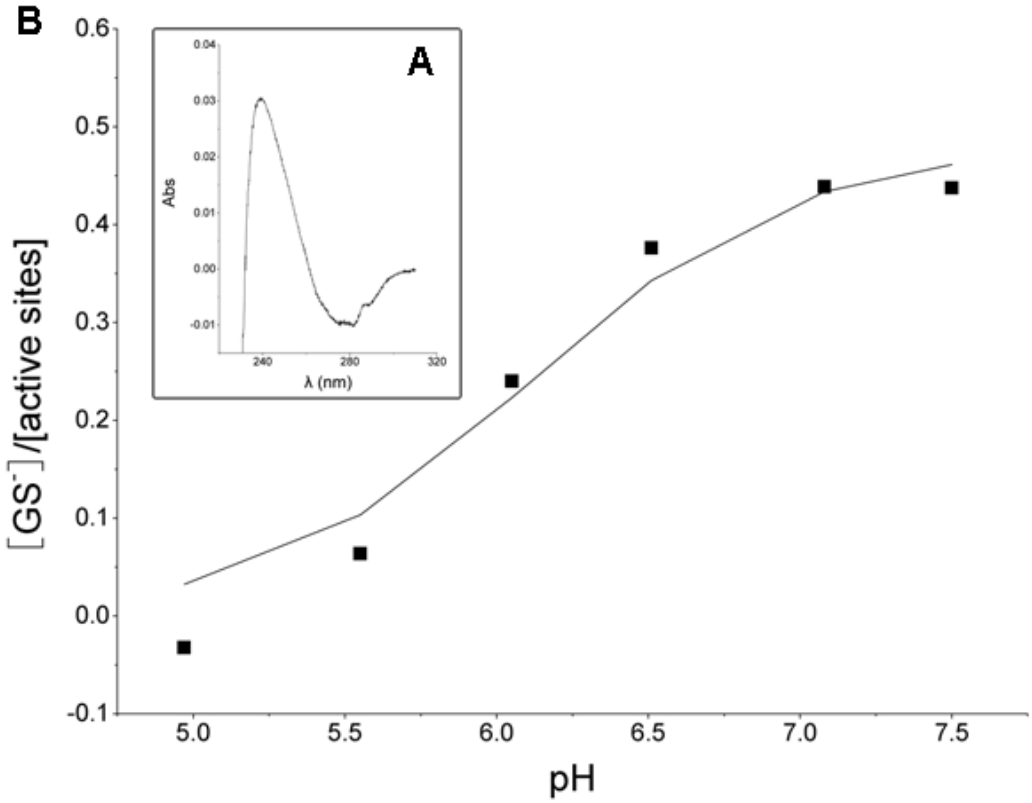

Figure S2

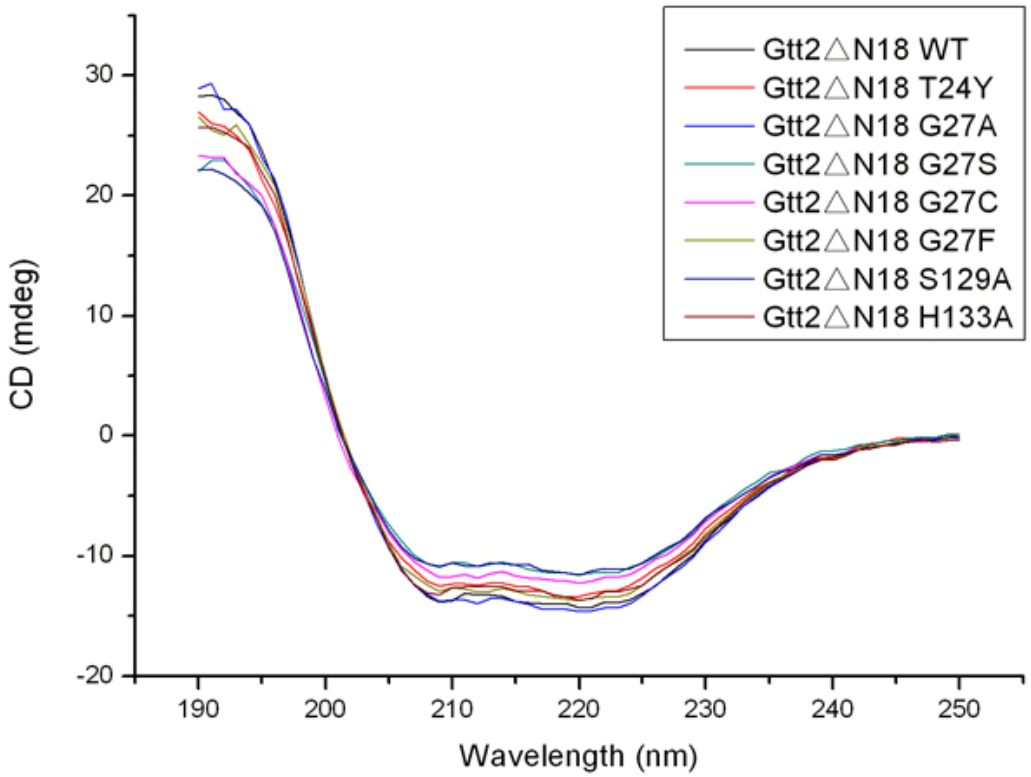
Gtt2△N18 WT
Gtt2△N18 T24Y
Gtt2△N18 G27A
Gtt2△N18 G27S
Gtt2△N18 G27C
Gtt2△N18 G27F
Gtt2△N18 S129A
Gtt2△N18 H133A
CD (mdeg)
30
20
10
0
-10
-20
190
200
210
220
230
240
250
Wavelength (nm)

Figure S3