



**SUPPLEMENTARY FIG. S3. Analysis of low-molecular-mass thiol binding to WT and  $\Delta 76$  *Tb1-C-Grx1*.** (A) Titration of WT *Tb1-C-Grx1* with GSH followed by intrinsic fluorescence. Fluorescence emission spectra ( $\lambda_{exc} = 295$  nm) of  $2.5 \mu\text{M}$  prereduced WT 1-C-Grx1 in 20 mM Tris-HCl pH 7.8, titrated with GSH up to  $27 \mu\text{M}$  (2–20 mM GSH stock solution freshly prepared in the same buffer). RFU: relative fluorescence units. Average spectra ( $n = 3$ ) are shown. The inset provides the emission intensities at the center of mass ( $\sim 350$  nm, arrow) as a function of the  $[\text{GSH}]/[\text{protein}]$  ratio using the color code of the figure. (B) Titration of WT *Tb1-C-Grx1* with GSH followed by CD. Near-UV CD spectra (250–320 nm) of  $70.2 \mu\text{M}$  *Tb1-C-Grx1* WT in 20 mM sodium phosphate, pH 7.4, titrated with GSH up to  $727 \mu\text{M}$  (2–20 mM GSH stock solution freshly prepared in the same buffer). Inset: plot of ellipticity at the wavelengths indicated with colored dots in the CD spectra versus  $[\text{GSH}]$ . (C) Structure of  $\Delta 76$  *Tb1-C-Grx1* with residues (sticks) shown to be involved in GSH binding in orthologous proteins (see main text and Fig. 7) and the single Trp residue (Trp142). The image was prepared with PyMOL based on PDB 2ltk, reported in this work. (D) Titration of  $\Delta 76$  *Tb1-C-Grx1* with low-molecular-mass thiol (LMM) ligands by NMR. Superposition of the  $^{15}\text{N}$ -HSQC spectra of  $\Delta 76$  *Tb1-C-Grx1* [0.4 mM protein in 50 mM sodium phosphate buffer, pH 7.0, 150 mM NaCl, and 10 mM DTT in 90:10% v/v  $\text{H}_2\text{O}/\text{D}_2\text{O}$  in free form (red contours) and upon addition of GSH at a protein:thiol ratio of 1:100 (left panel, blue contours) or  $\text{T}(\text{SH})_2$  at a protein:thiol ratio of 1:50 (right panel, blue contours)]. All spectra were recorded under reducing conditions (10 mM DTT). The assignment for the residues forming the GSH-binding pocket and highlighted with colors in Figure 7 is shown. Peaks arising from unlabeled GSH and  $\text{T}(\text{SH})_2$  present at a high concentration in the sample are indicated with black arrows. (E) Titration of WT *Tb1-C-Grx1* with LMM thiol ligands by NMR. Representative titration curves of 0.3 and 0.2 mM *Tb1-C-Grx1* titrated with GSH and  $\text{T}(\text{SH})_2$ , respectively. GSH, glutathione; CD, circular dichroism; NMR, nuclear magnetic resonance; HSQC, heteronuclear single-quantum coherence.