Supporting Information

Redox-Inactive Peptide Disrupting Trx1-Ask1 Interaction for Selective Activation of Stress Signaling

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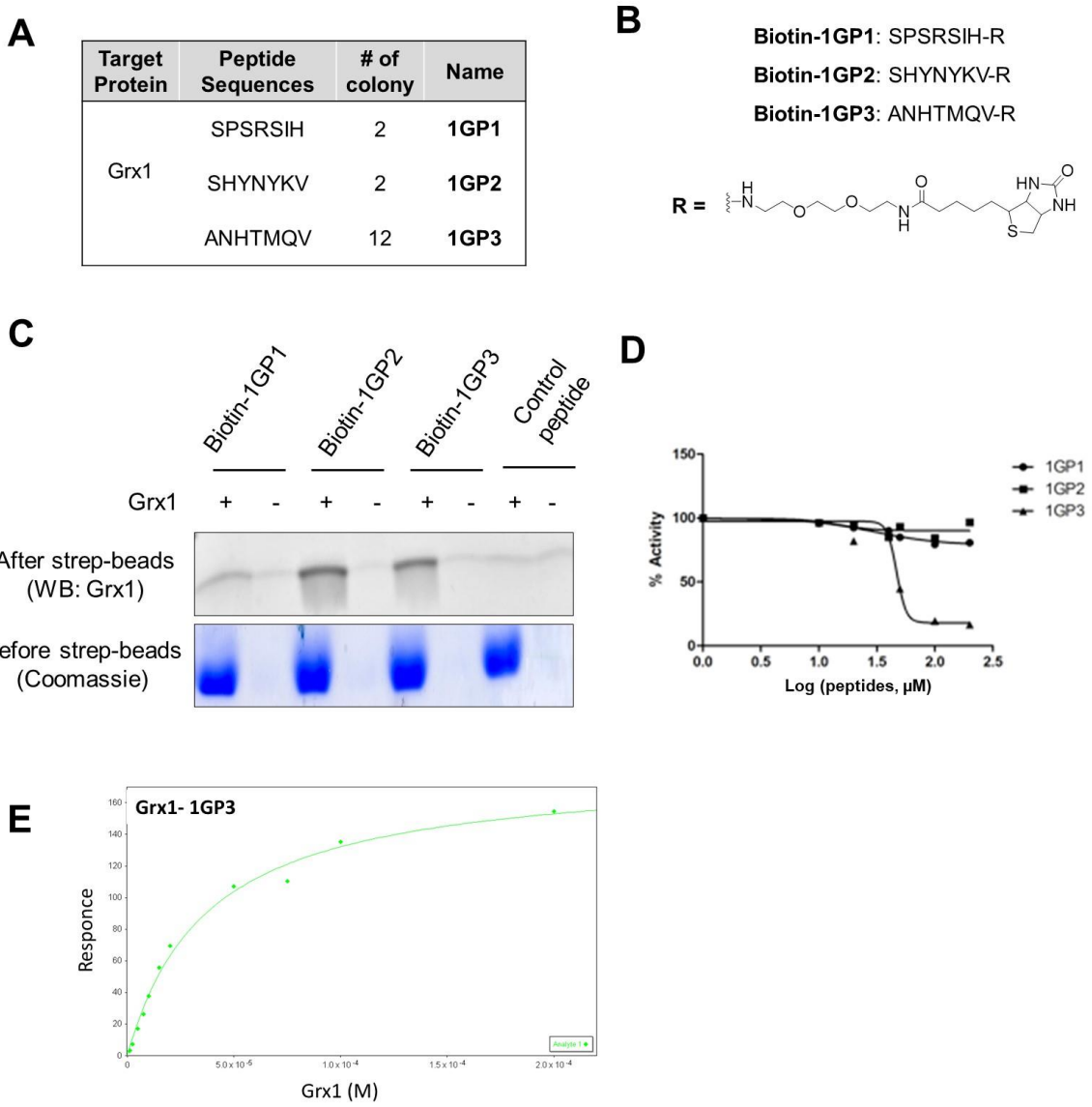

Figure S1. In vitro evaluation of 1GP3 with Grx1. (A) Peptide sequences (1GP1, 1GP2, 1GP3) identified from phage library screening with Grx1. (B) Structure of biotinylated 1GP1, 1GP2, and 1GP3. (C) Binding analyses of 1GP1, 1GP2, and 1GP3 with Grx1. Biotin-1GP peptides were bound on streptavidin beads, and incubated with purified Grx1. A control peptide was made as follows: The C-terminus of human RNR (C^{830}SLENRDEC^{838}LMC^{841}GS) was modified to have Ser in place of C830/838/841 and a biotin group on a N-terminus, resulting in the control peptide (biotin-S^{830}SLENRDES^{838}LMS^{841}GS). (D) Inhibitory activity of 1GP peptides for Grx1 deglutathionylation. (E) binding affinity of 1GP3 to Grx1 measured by SPR.

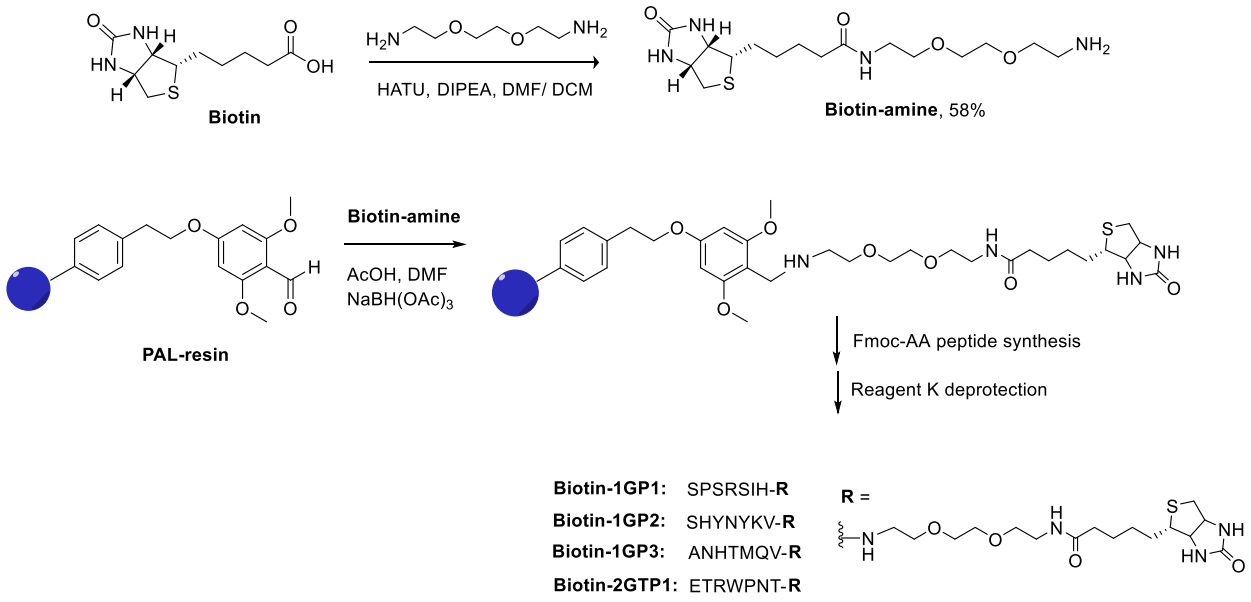

Figure S2. A synthetic scheme of biotinylated peptides 1GP1, 1GP2, 1GP3, and 2GTP1.

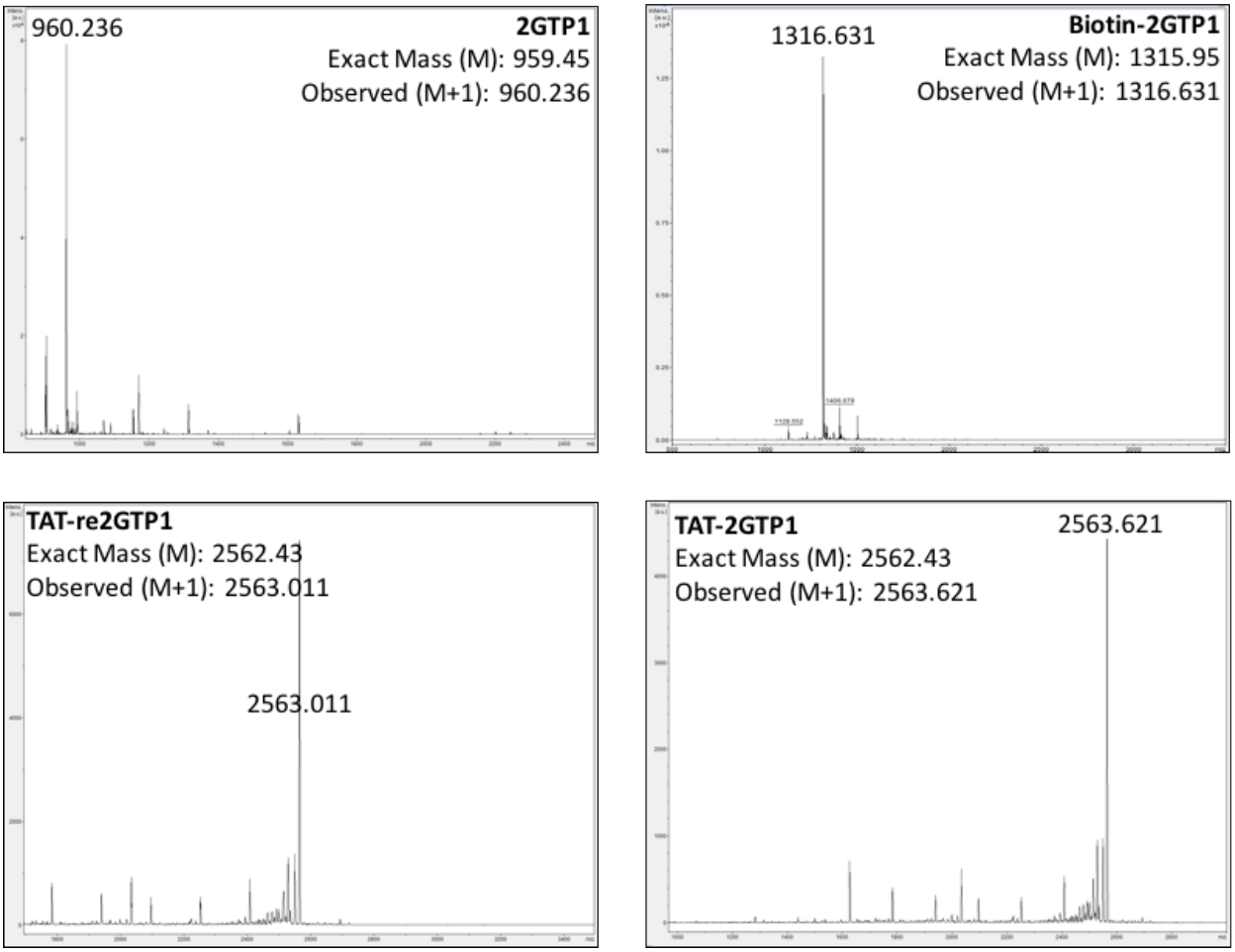

Figure S3. MALDI mass spectrum of synthesized peptides, 2GTP1, biotin-2GTP1, TAT-2GTP1, and TAT-re-2GTP1.

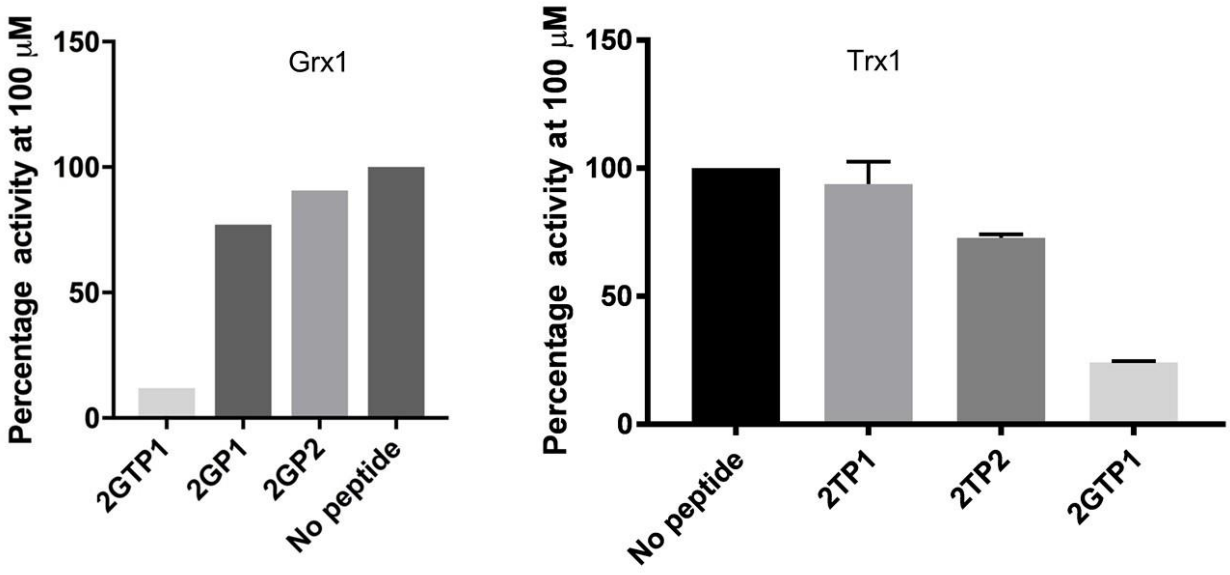

Figure S4. An initial evaluation of peptides identified from bio-panning with Grx1 or Trx1 (2nd and 3rd trials). De-glutathionylation of glutathionylated-Cys (GS-Cys) by Grx1 was measured in a GR-coupled enzyme assay by monitoring NADPH oxidation at 340 nm. The initial rates in the presence of individual peptide (100 μM) were compared to the rate without any peptide, and shown as the percentage activity (left). Insulin reduction-induced aggregation by Trx1 was measured at a 10-min time point by UV at 650 nm in the presence of individual peptide (all 100 μM), and shown as percentage activity versus one without a peptide (right).

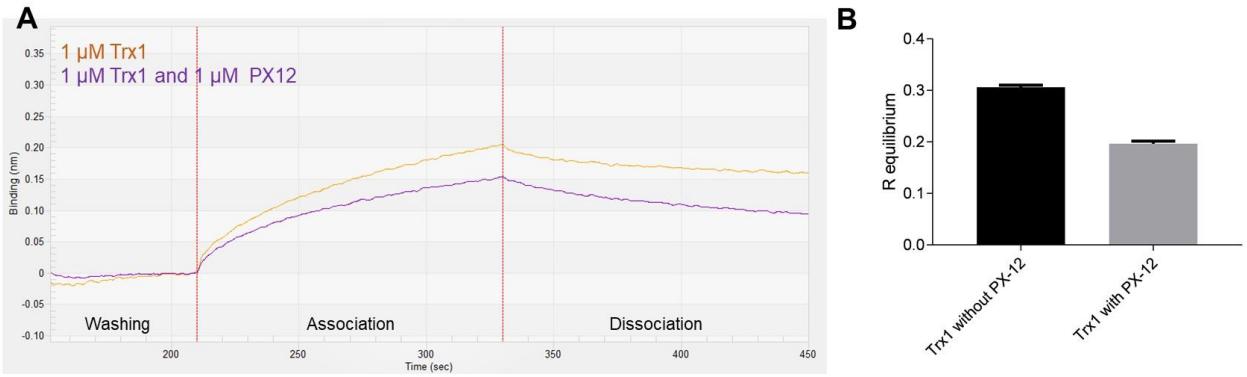

Figure S5. Binding analyses of 2GTP1 to Trx1 in the presence of PX-12. (A) The BLI sensorgram that shows the plot of the response (binding) upon addition of Trx1 in the presence or absence of PX-12 against time. The streptavidin-coated sensor was immobilized with biotin-2GTP1 for 150 sec (not shown) and incubated in sequence in following solutions in buffer A containing Tris-HCl (100 mM), NaCl (150 mM), EDTA (3 mM), Tween-20 (0.005%) and DTT (1 mM): 1) buffer A (150-210 sec), 2) Trx1 (1 µM) in buffer A with or without PX-12 (1 µM) (210-340 sec), and 3) buffer A (340-450 sec). Note the presence of DTT in a binding assay, which is necessary to see binding of 2GTP1 to Trx1, but could compromise the effect of PX-12. (B) The response equilibrium (R equilibrium) that represents the bound concentration is shown. The response is normalized to a condition with Trx1 in the absence of PX-12.

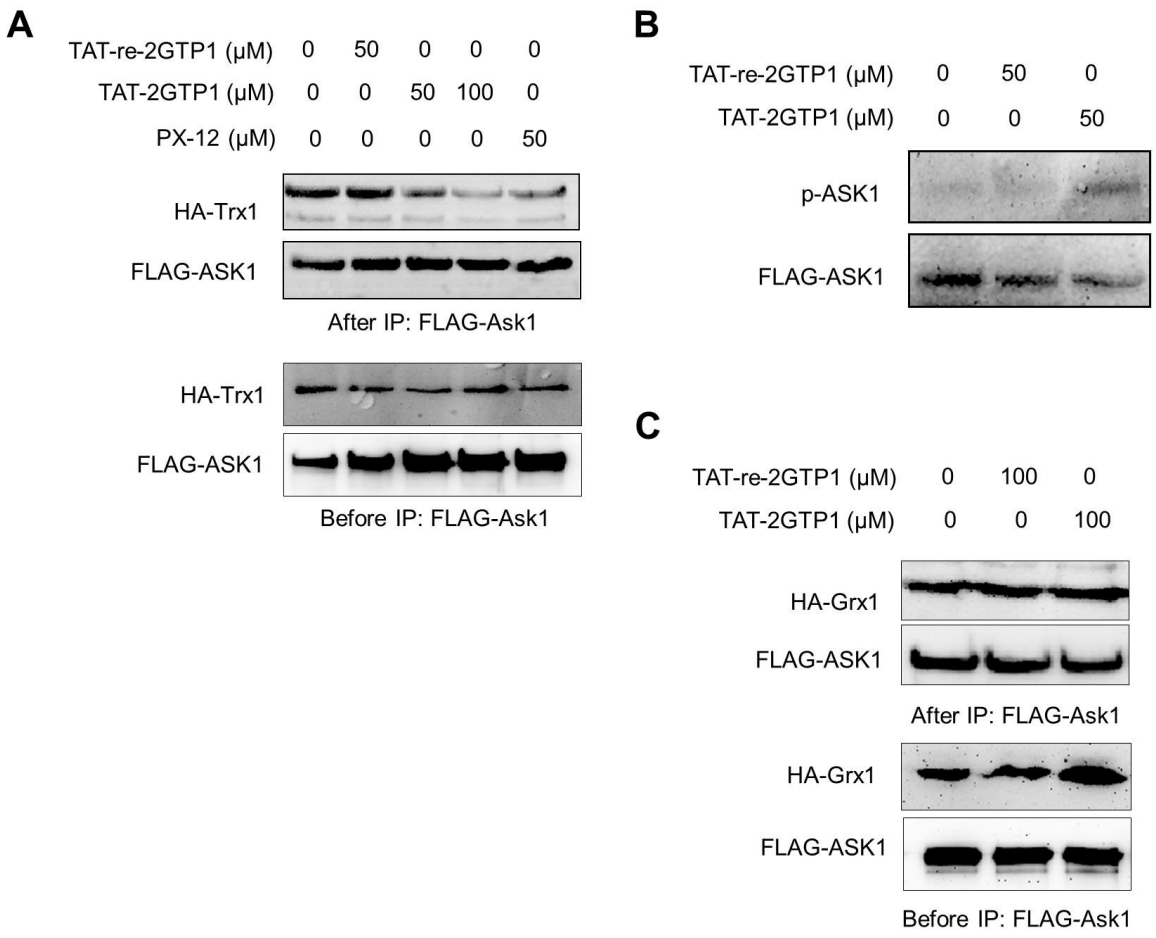

Figure S6. Cellular evaluation of TAT-2GTP1 for activating Ask1 and disrupting Trx1-Ask1 and Grx1-Ask1 interaction. (A) Ask1-Trx1 interaction analyzed by co-IP in MDA-MB-231 cells. After transfection of FLAG-Ask1 and HA-Trx1 to cells, TAT-2GTP1, TAT-re-2GTP1, or PX-12 was incubated for 6 h. (B) Ask1 phosphorylation upon incubation of TAT-2GTP1 in HEK293 cells for 6 h. (A) Ask1-Grx1 interaction analyzed by co-IP in MDA-MD-231 cells. After transfection of FLAG-Ask1 and HA-Grx1 to cells, TAT-2GTP1 or TAT-re-2GTP1 was incubated for 6 h.

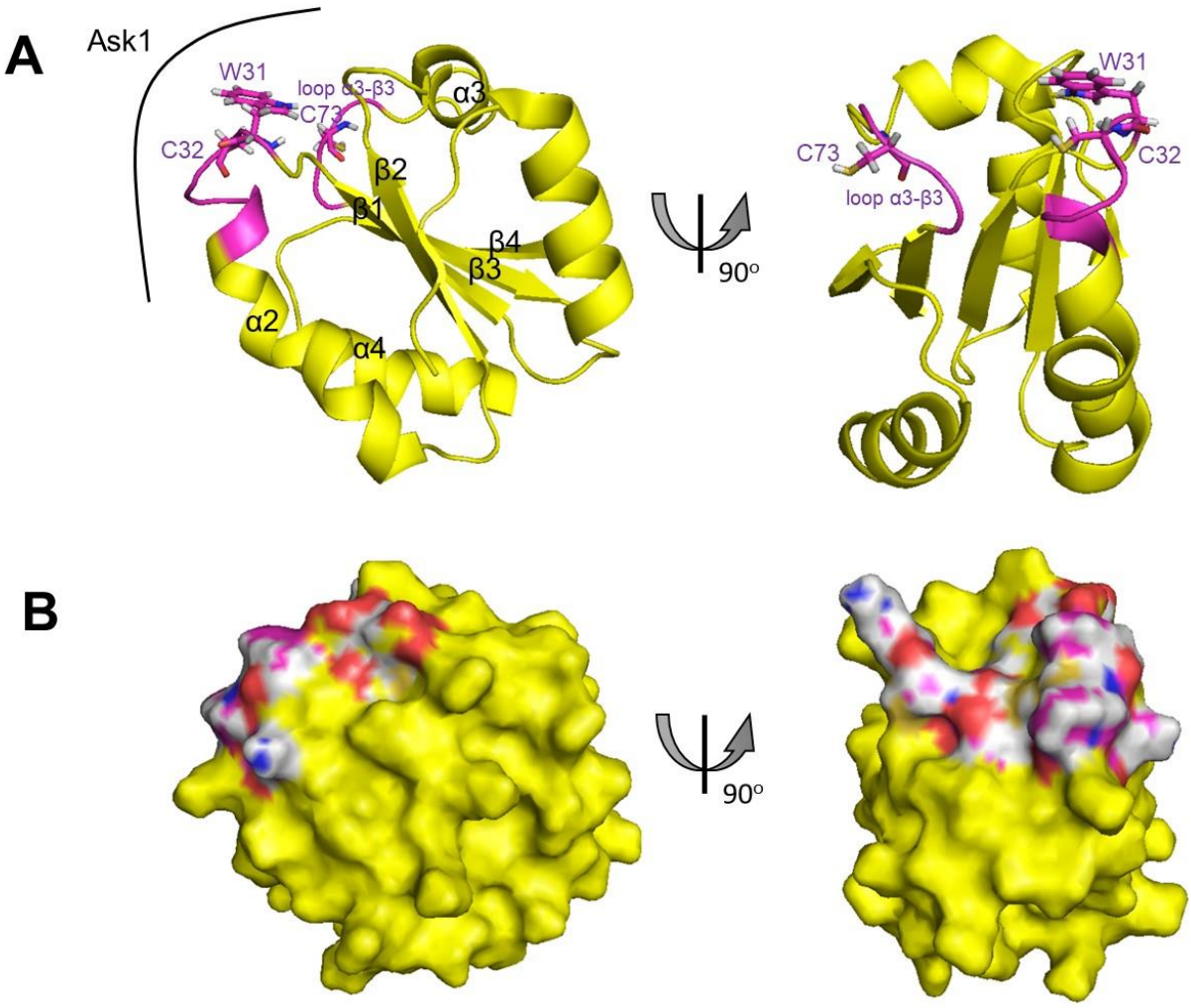

Figure S7. The potential amino acids in the binding interface between Trx1 and a thioredoxin-binding domain of Ask1 (Ask1-TBD). The literature[S4-S5] suggests that non-covalent interactions between Trx1 and Ask1-TBD in a reducing condition are mediated by a relatively broad surface area in Trx1 that includes a ^{31}WCGPC^{35} motif containing Cys32 and a loop connecting an α3 helix and a β3 strand (loop α3-β3) containing Cys73. (A) A ribbon diagram of Trx1 that highlights a ^{31}WCGPC^{35} motif and a loop α3-β3 in magenta. (B) The surface contour of Trx1 structure shown in Figure S7A. The structures are drawn from PDB 3TRX with the available information.[S4-S5]

Material and Methods

General. Cell culture reagents were purchased from Life technologies. All other reagents and solvents were purchased from Sigma-Aldrich.

Expression of proteins. Expression and purification of Grx1[S1], Trx1 and Grx3[S2] were reported previously.

Peptide Synthesis.

Biotinylated peptide synthesis (biotinylated 1GP1, 1GP2, 1GP3, and 2GTP1). Biotin-amine was synthesized as reported previously.[S3] Biotin-amine (2.5 eq.) in DMF was added to DFPE (aldehyde) polystyrene resin, followed by addition of $NaBH(OAc)_3$ (8 eq.) and 2% acetic acid. The mixture was incubated overnight at room temperature, and the beads were washed with DMF and dichloromethane. After coupling biotin-amine to the resin, a typical solid-state peptide synthesis was carried out, using Fmoc amino acid peptide chemistry. HATU was used as the activator, and DIPEA was used as a base. The cleavage of the peptides from the resin was done by Reagent K with or without 1,2-ethanedithiol. Cleaved peptides were purified by HPLC, and the mass was confirmed by MALDI.

TAT-tagged peptide synthesis (TAT-2GTP1, TAT-re-2GTP1, TAT). Gly-pre-loaded Wang resin was used to synthesize TAT (GRKKRQRRRPQ)-containing peptides with Fmoc amino acid peptide chemistry. HATU was used as the activator and DIPEA was used as the base. One Gly residue was introduced between TAT-tag and 2GTP1 or re-2GTP1. The cleavage of the peptides from the resin was done by Reagent K with or without 1,2-ethanedithiol. Cleaved peptides were purified by HPLC, and the mass was confirmed by ESI or MALDI.

Cell culture. Human embryonic kidney (HEK) 293, MDA-MB-231, MCF7 and MDA-MB-453 cells were obtained from ATCC, and grown in high glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with heat inactivated 10% fetal bovine serum (FBS) and 5 U/mL of penicillin and 5 µg/mL of streptomycin, and maintained in a humidified incubator in 37°C at 5% CO_2.

References

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