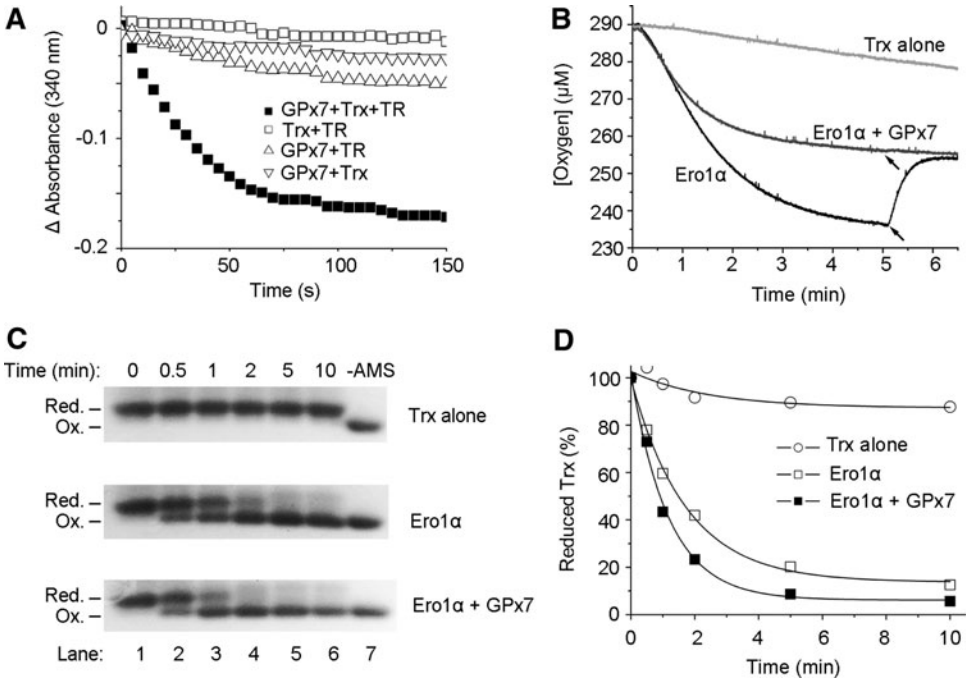
Supplementary Data

References

1. Appenzeller-Herzog C, Riemer J, Christensen B, Sorensen ES, and Ellgaard L. A novel disulphide switch mechanism in Ero1 alpha balances ER oxidation in human cells. *EMBO J* 27: 2977–2987, 2008.
2. Wang X, Wang L, Wang Xe, Sun F, and Wang CC. Structural insights into the peroxidase activity and inactivation of human peroxiredoxin 4. *Biochem J* 441: 113–118, 2012.



SUPPLEMENTARY FIG. S1. Glutathione peroxidase 7 (GPx7) exhibits TGPx activity and oxidizes thioredoxin by using Ero1α-derived hydrogen peroxide (H_2O_2). (A) NADPH (0.15 m*M*) oxidation was carried out at 25°C by monitoring absorbance changes at 340 nm with addition of 40 μM H_2O_2, in the presence or absence of 10 μM *Escherichia coli* thioredoxin (Trx), 10 μM thioredoxin reductase (TR), and 10 μM GPx7, as indicated (2). **(B)** Oxygen consumption was monitored at 25°C, as reduced Trx (50 μM) was re-oxidized alone or in the presence of 2 μM Ero1α, or combined with 10 μM GPx7. Catalase was added at the indicated time points (*arrows*). Without GPx7, ~45 μM O_2 was consumed to oxidize 50 μM Trx, and ~40 μM H_2O_2 can be detected on catalase added; with GPx7, 25 μM O_2 was consumed and no H_2O_2 was detected at the end. **(C)** Parallel experiments were carried out as the same in **(B)**, and at indicated time points, aliquots were taken out for quenching using 2 m*M* 4-acetamido-4′-maleimidylstilbene-2,2′-disulfonic acid (AMS). The samples were analyzed by sodium dodecyl sulfate-15% polyacrylamide gel electrophoresis (SDS-15% PAGE) and visualized by Coomassie staining to determine the reduced (Red.) and oxidized (Ox.) Trx. Reduced Trx without AMS treatment (-AMS) was loaded as control. **(D)** The reduced fractions at each time point in **(C)** were quantified by densitometry using ImageJ software and plotted as the percent remaining relative to 0 min.