

Figure S1. PrxIV C51A has peroxidase activity *in vitro*.

Peroxiredoxin activity was assayed as previously described (Tavender & Bulleid, 2010) with 4.5 μM PrxIV (WT, C51A or DM), 3 μM thioredoxin, 1.5 μM thioredoxin reductase, 100 μM hydrogen peroxide and 200 μM NADPH in 50 mM HEPES pH 7. NADPH consumption was recorded spectrophotometrically at 340 nm. Negative control reaction 'Neg' has no PrxIV added.

Figure S2. Wild-type PrxIV comprises a complex mix of disulphide- linked multimers.

PrxIV (3 μM) was incubated with 10 mM GSH as indicated then TCA added and precipitates re-dissolved in SDS sample buffer plus NEM. Half of each sample was subjected SDS-PAGE under non-reducing conditions (top panel) and half under reducing conditions (bottom). Both were visualised by Coomassie blue staining. Gel positions of different redox forms are indicated.

Figure S3. Reduction of purified PDI family members.

DTT (5 mM) was added to ERp46, P5, PDI, ERp57 and ERp18, and samples incubated for 15 minutes and then DTT was removed by buffer exchange. Samples of each were treated with TCA and precipitates were re-dissolved in denaturing buffer containing AMS. Each was visualised by SDS-PAGE (reducing conditions) and Coomassie blue staining (lane 3). Reduced (lane 1) and oxidised (lane 2) controls were prepared by incubation of each protein with 20 mM DTT or 1 mM DPS respectively, for 15 minutes prior to TCA addition. Gel positions of reduced (red) and oxidised (ox) species are indicated.

Figure S4. Quantification of ER resident proteins in HT1080 cells.

(A) HT1080 cells were lysed in SDS sample buffer at a concentration of 10^4 cells / μl . Samples equivalent to 10^5 cells were then developed alongside specified quantities of each purified protein using fluorescent Western blots. (B) Densitometry was used to plot intensity

of samples against quantity of protein present for each titration of purified protein. Using these data, protein content was calculated for cell lysates and expressed as picomoles of each protein contained within 10^6 HT1080 cells. Data presented are mean average values (\pm SD) determined from duplicate analyses of three independent lysates (A, B and C). Endogenous ERp18 could not be detected in lysates so was determined to be present at quantities less than 2.5 ng per 10^5 cells.

Figure S1

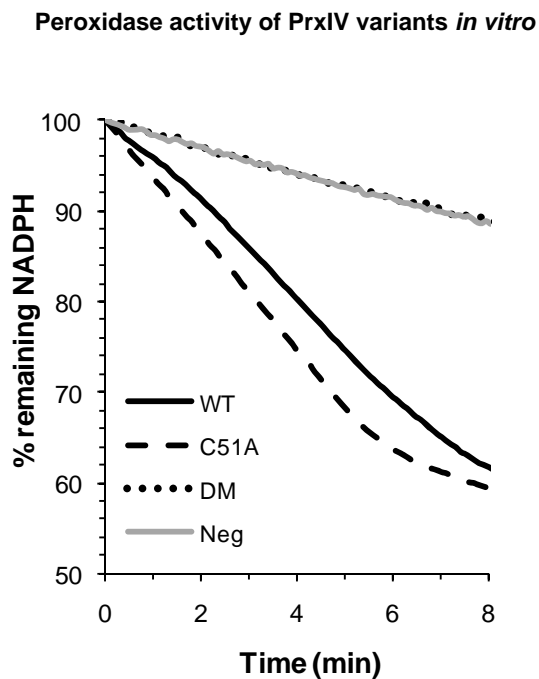


Figure S2

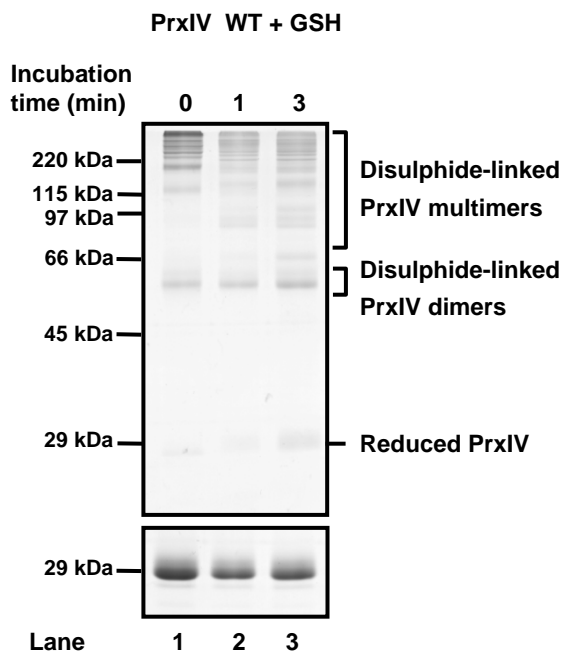


Figure S3

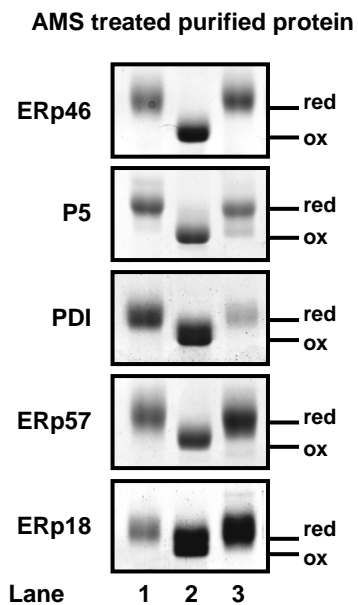


Figure S4

