Supplementary material

The intrinsically disordered membrane protein Selenoprotein S is a reductase *in vitro*

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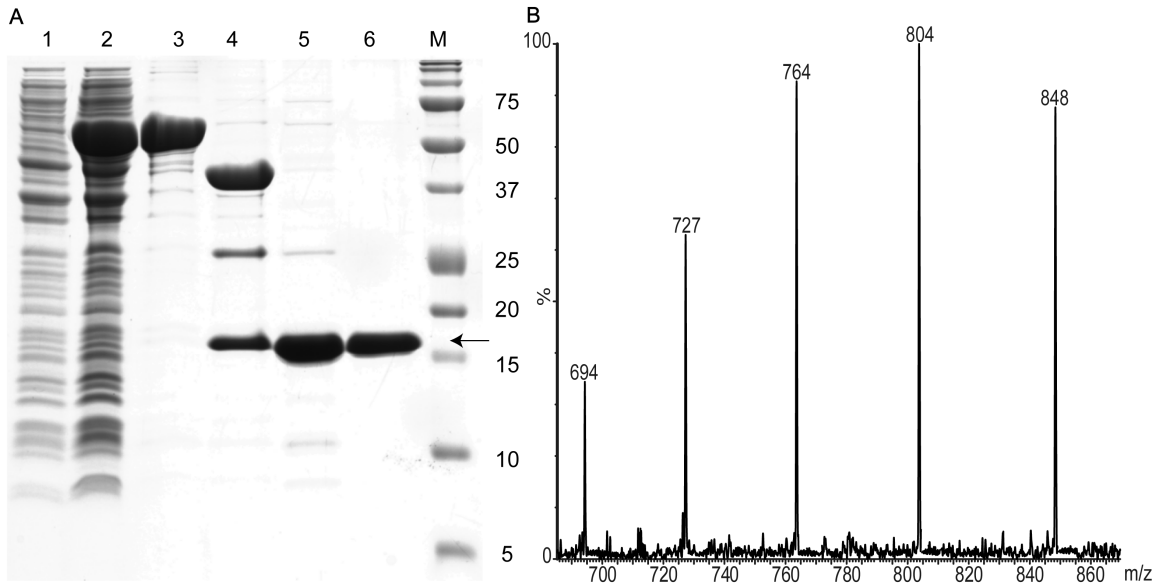

Figure S1. Expression and purification of cSelS U188C. (A) SDS-PAGE analysis. Lane 1: Crude cell extract prior to induction. Lane 2: Crude cell extract after overnight expression at 18 °C. Lane 3: IMAC elute. Lane 4: TEV protease cleavage mixture. Lane 5: cSelS U188C after TEV protease and MBP were removed by cation exchange chromatography. Lane 6: cSelS U188C (indicated by an arrow) following purification with sephacryl S-100. M: Unstained protein molecular weights standard (the molecular mass in kDa is noted on the right). (B) The molecular weight of cSelS U188C detected by electrospray ionization mass spectrometry is 15252 Da (predicted molecular weight is 15253 Da).

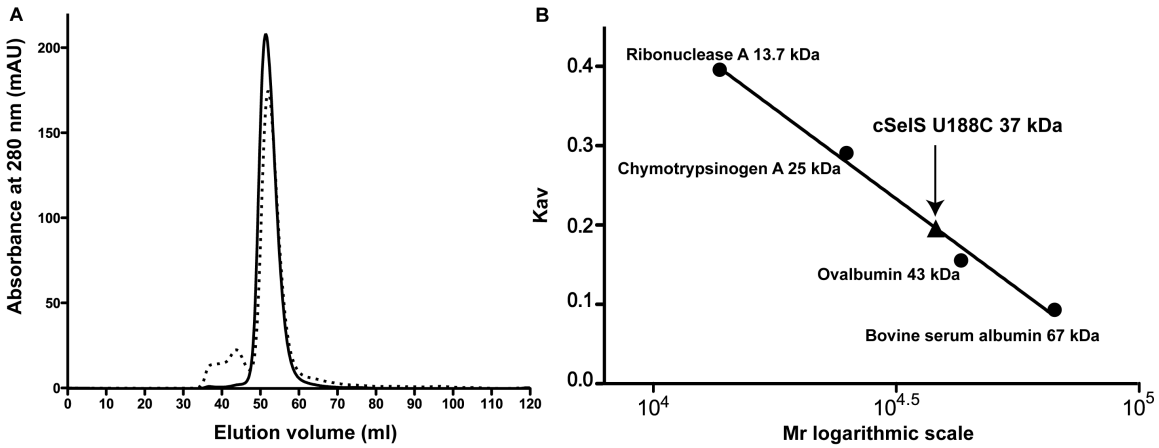

Figure S2. Characterization of cSelS U188C by size-exclusion chromatography. (A) cSelS U188C eluted from sephacryl S-100 column in the presence (solid line) and absence of the reducing agent DTT (dotted line). (B) The apparent molecular weight of the cSelS U188C was determined by measuring its elution volume relative to standard proteins. The black triangle overlaid on the calibration curve corresponds to the apparent molecular weight of 37 kDa for cSelS U188C. Hence, SelS U188C is a dimer.

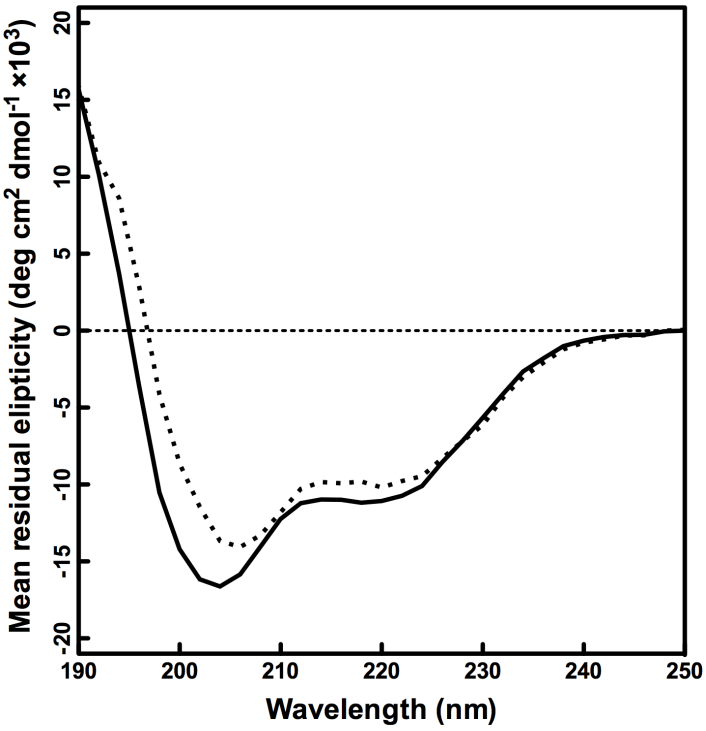

Figure S3. CD spectra of cSelS (dotted line) and cSelS U188C (solid line). CD spectra were acquired at 20 °C in 10 mM potassium phosphate buffer (pH 7.5) and 50 mM sodium sulfate.

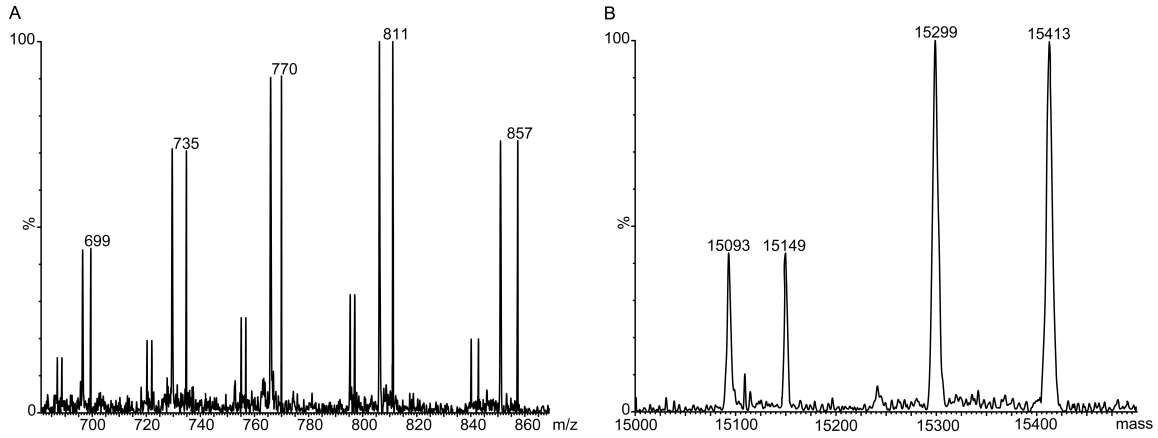

Figure S4. Ionization efficiencies of oxidized cSelS and reduced and alkylated cSelS by electrospray ionization mass spectrometry. Mass spectra of 1:1 mixture of oxidized cSelS (15299 Da) and reduced and alkylated cSelS (15413 Da). Smaller peaks correspond to the oxidized cSelS 188Δ (15093 Da) and reduced and alkylated cSelS 188Δ (15149 Da). (A) m/z spectrum. (B) Deconvoluted spectrum. The proteins' ionization efficiencies are similar; hence, the mass spectrum reflects the relative ratios of the different forms of proteins in the sample.

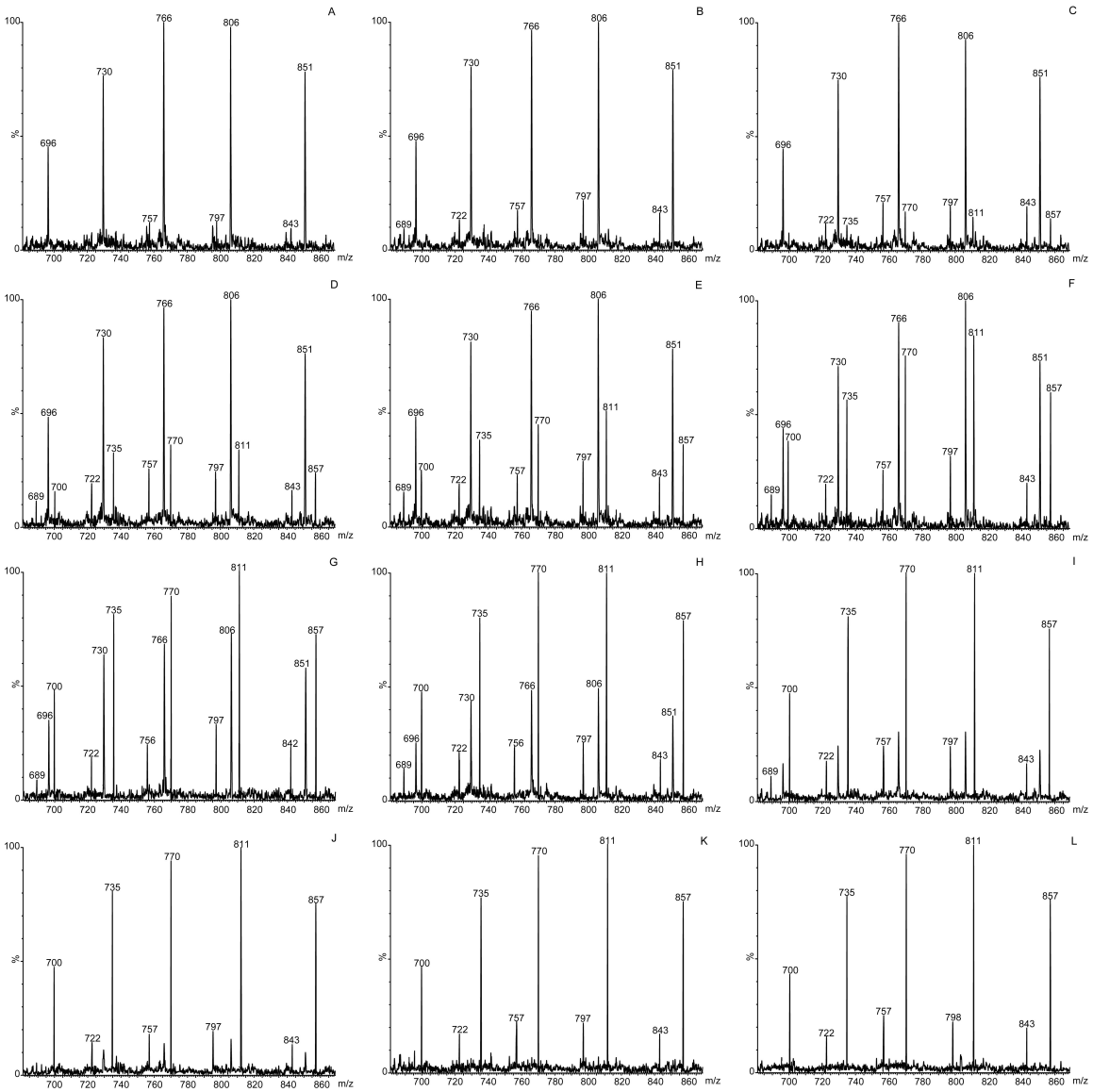

Figure S5. Electrospray ionization mass spectra of cSelS following incubation in various redox buffers and subsequent alkylation. Each panel shows the charge state distribution of multiply charged ions $[M+18H]^{18+}$ to $[M+22H]^{22+}$ from m/z 680 to 870. Detected molecular weights: cSelS 188Δ alkylated with one acetamide group on Cys174 at 15149 Da; oxidized cSelS at 15298 Da; cSelS labeled with two acetamide groups (on Cys174 and Sec188) at 15415 Da. The redox potential of the buffers is set at: (A) -171 mV. (B) -196 mV. (C) -211 mV. (D) -219 mV. (E) -226 mV. (F) -232 mV. (G) -237 mV. (H) -242 mV. (I) -248 mV. (J) -257 mV. (K) -272 mV. (L) -289 mV.

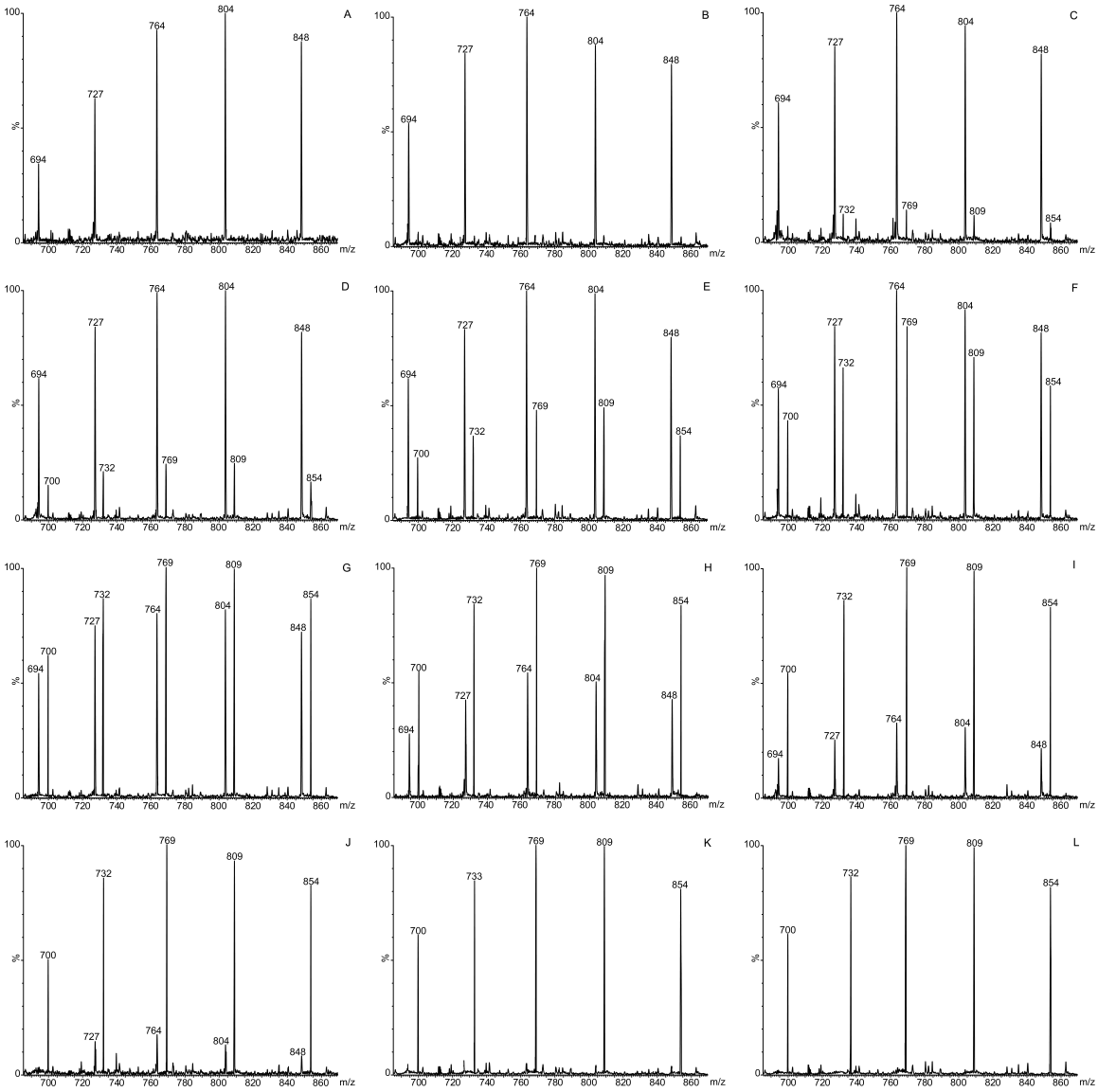

Figure S6. Electrospray ionization mass spectra of cSelS U188C following incubation in various redox buffers and subsequent alkylation. Each panel shows the charge state distribution of multiply charged ions $[M+18H]^{18+}$ to $[M+22H]^{22+}$ from m/z 685 to 870. Detected molecular weights: oxidized cSelS U188C at 15253 Da and cSelS labeled with two acetamide groups (on Cys174 and Sec188) at 15365 Da. The redox potential of the buffers are: (A) -142 mV. (B) -171 mV. (C) -189 mV. (D) -196 mV. (E) -203 mV. (F) -209 mV. (G) -213 mV. (H) -219 mV. (I) -226 mV. (J) -234 mV. (K) -249 mV. (L) -289 mV.

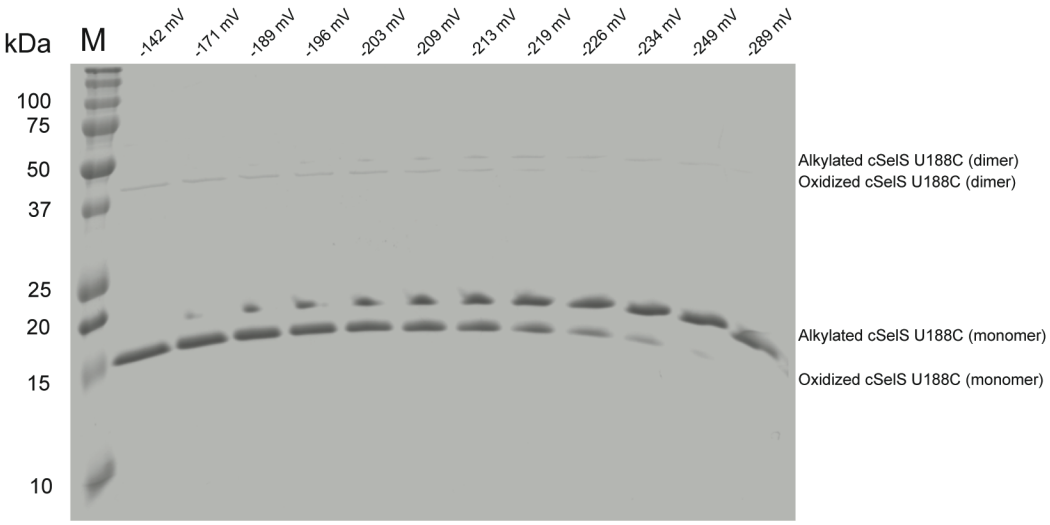

Figure S7. Determination of the reduction potential of cSelS U188C by gel shift assays using alkylation with $MM(PEG)_{24}$. Species were separated by SDS-PAGE. The protein bands are labeled to the right of the image to simplify identification. M denotes molecular weight markers (biorad Precision Plus Protein™ Dual Xtra Standards). The measured reduction potential is identical to that measured by mass spectrometry.

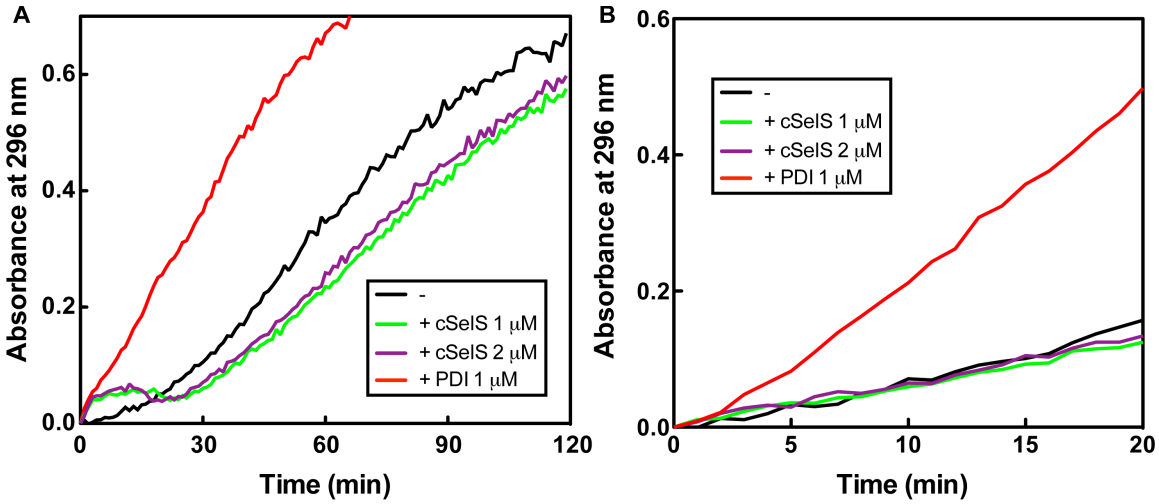

Figure S8. cSelS oxidase and isomerase activities were measured by monitoring the absorbance at 296 nm of RNase A-catalyzed hydrolysis of cCMP. Human protein disulfide isomerase (PDI) is used as a positive control. (A) Oxidase activity using reduced and denatured RNase A. (B) Isomerase activity using scrambled RNase A. cSelS has no oxidase or isomerase activities.

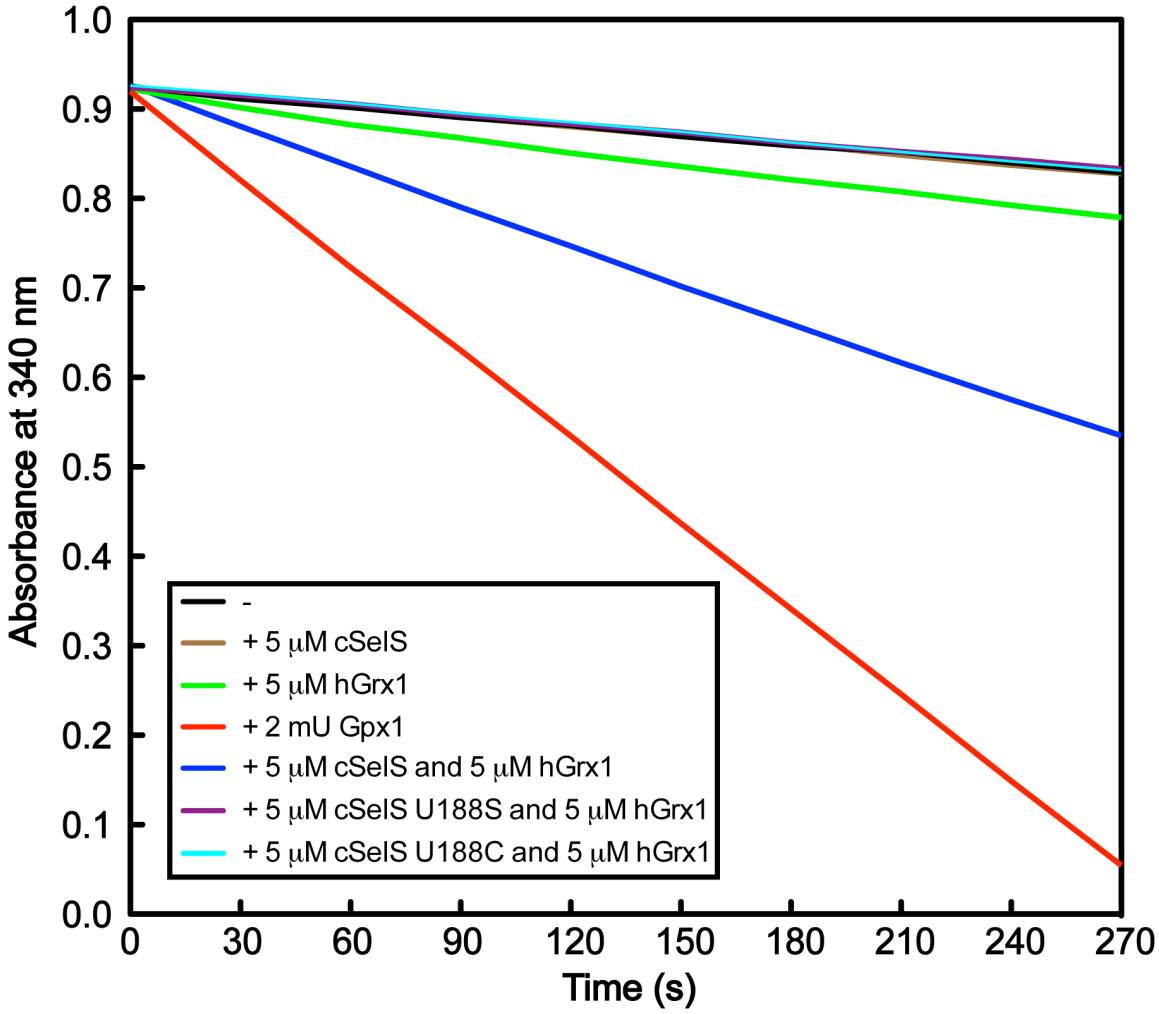

Figure S9. cSelS peroxidase activity assayed with human glutaredoxin 1 (hGrx1) and yeast glutathione reductase. Glutathione peroxide (GPx1) from bovine erythrocytes was used as a positive control. The rate of NADPH oxidation in the presence of hGrx is about half of that in the presence of equal concentrations of hTrx.