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Electronic supplementary information

Dimers of Glutaredoxin 2 as Mitochondrial Redox Sensors in Selenite-induced Oxidative Stress

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Fig. S 1. Determination of protein-bound glutathione

Fig. S 2. Determination of Grx2 monomerization in cells after oxidative stress induction

Fig. S 3. Specific activity of TrxR1 and TrxR2 and of cytosolic and mitochondrial Grx in Sel treated cells

Fig. S 4. Iron distribution in the two mitochondrial fractions obtained after treatment with *meta*-phosphoric acid

Fig. S 5. FACS analysis of lipid peroxidation in HeLa cells treated with Sel +/- BHT using C11-BODIPY dye

Fig. S 6. Release of Cyt C after Sel treatment in HeLa cells.

Fig. S 7. Western blot analysis of GPx4 in mitochondria samples in the presence of 15 μM Sel.

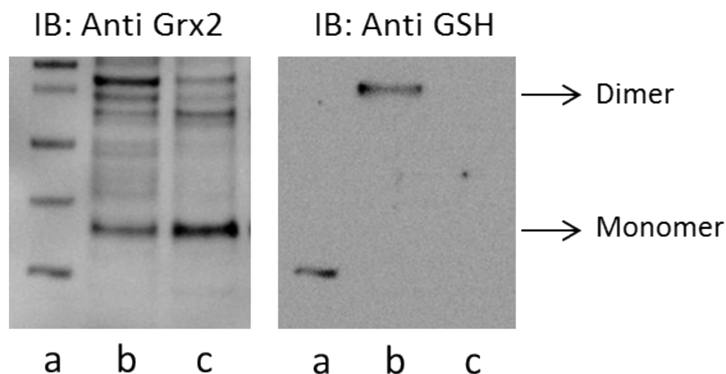

Figure S 1. Determination of protein-bound glutathione. HeLa cells were treated for 18 h with 15 μM Sel. To analyze the monomerization of Grx2, mitochondrial cell fractions derivatized with 10 mM AIS, were subjected to Nu-PAGE in non-reducing conditions and WB analysis. The membrane was probed with an anti-Grx2 and an anti-GSH Ab. (a) Markers; (b) Cnt; (c) 15 μM Sel. (IB: immunoblot).

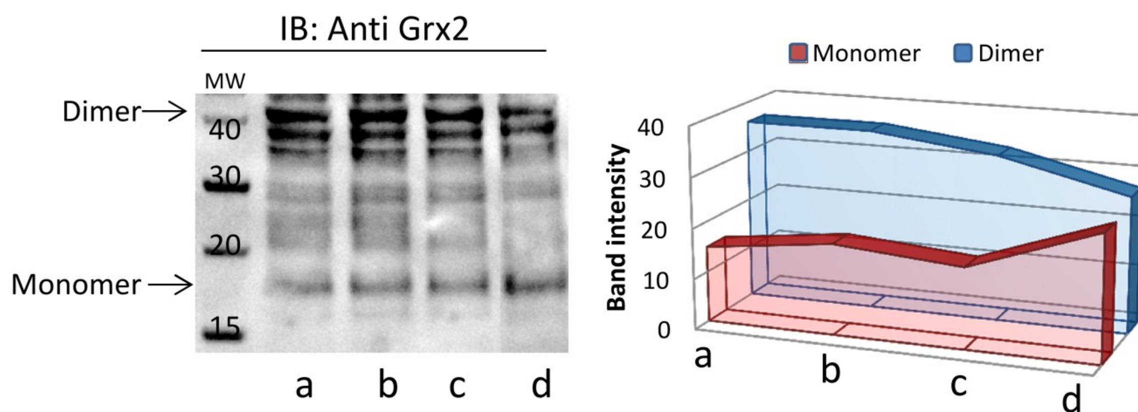

Figure S 2. Determination of Grx2 monomerization in cells after oxidative stress induction. HeLa cells were treated with **a**: Cnt; **b**: 1 mM BSO 48 h; **c**: 1 μM AF 3 h, **d**: 1 mM BSO 48 h + 1 μM AF 3 h. To analyze the monomerization of Grx2, mitochondrial cell fractions, derivatized with 10 mM AIS, were subjected to SDS-PAGE in non-reducing conditions and WB analysis. The densitometric analysis of the bands performed with NineAlliance software is reported in the right panel.

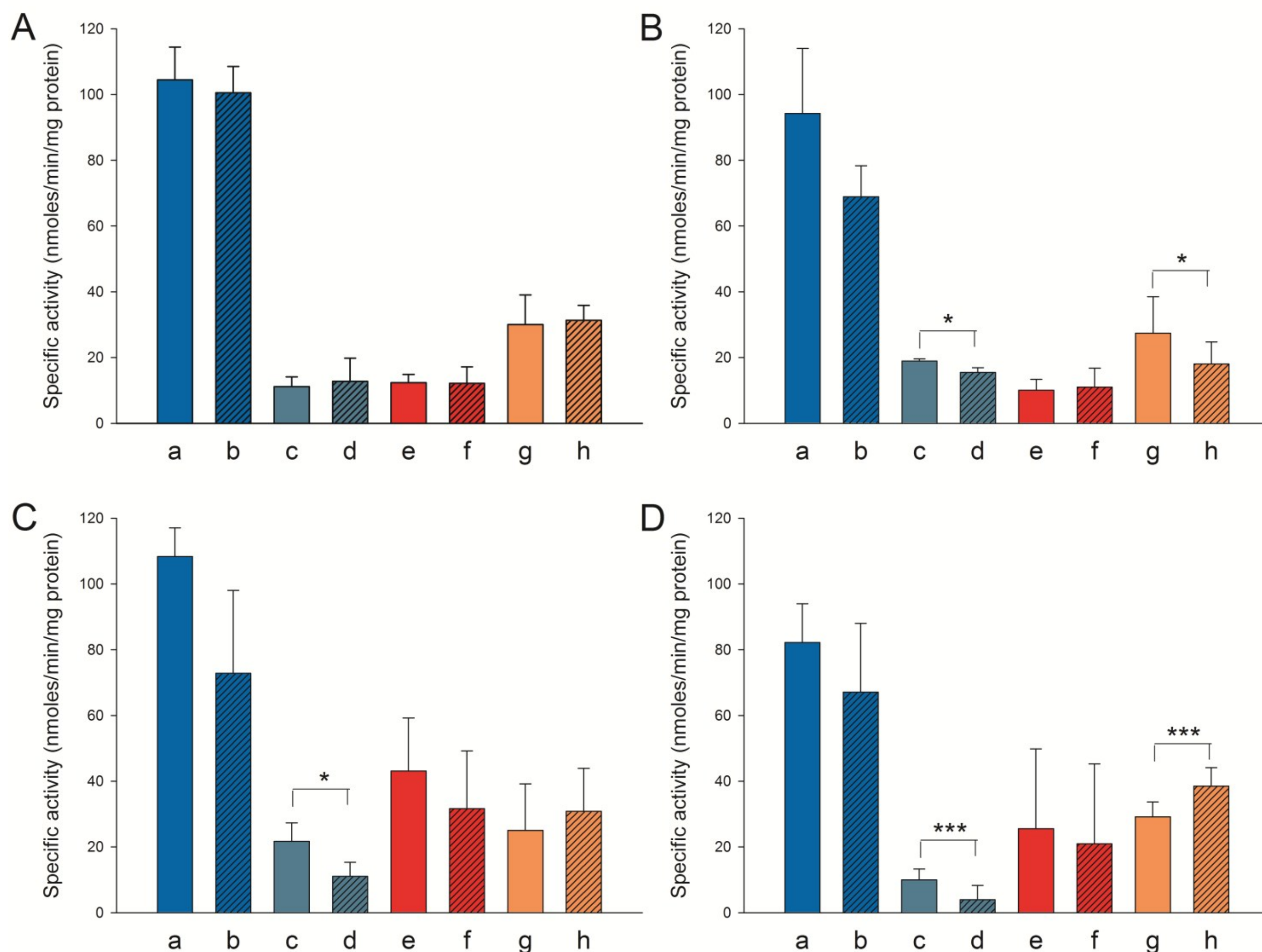

Figure S 3. Specific activity of TrxR1 and TrxR2 and of cytosolic and mitochondrial Grx in Sel treated cells. HeLa cells (3 x 10^7) were incubated for 3 (**A**), 6 (**B**), 12 (**C**) and 18 (**D**) h with 15 μM Sel or in control conditions and then subjected to cell sub-fractionation in order to obtain the cytosol and mitochondria enriched cell fractions. The cytosolic fraction was checked for TrxR1 (blue bars) and total glutaredoxin (red bars) activities; the mitochondrial fraction was probed for TrxR2 (gray bars) and Grx2 (orange bars) activities. The graphs reports the specific activity of each enzyme, expressed as nmoles/min/mg protein, in Sel treated or control cells. **a**: TrxR1 Cnt; **b**: TrxR1 15 μM Sel; **c**: TrxR2 Cnt; **d**: TrxR2 15 μM Sel; **e**: cytosolic Grx Cnt; **f**: cytosolic Grx 15 μM Sel; **g**: mitochondrial Grx Cnt; **h**: mitochondrial Grx 15 μM Sel. The graph shows the mean ± SD of 3 experiments (*$p<0.05$, ***$p<0.001$).

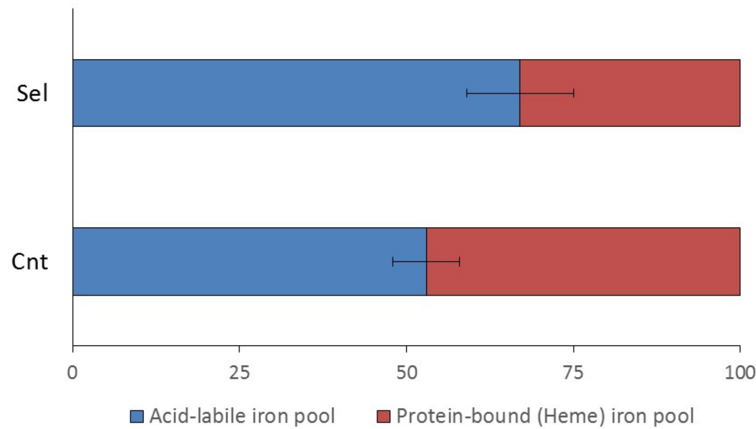

Figure S 4. Iron distribution in the two mitochondrial fractions obtained after treatment with *meta*-phosphoric acid. HeLa cells (3 x 10^7) were treated with 15 µM Sel for 18 h and then processed to obtain mitochondria following the protocol of Clayton and Shadel (see Experimental section). Mitochondria were then treated with 600 µL of 6% *meta*-phosphoric acid for 20 min at 4°C in order to extract the labile iron pool. At the end of incubation, samples were centrifuged at 15800g for 10 min at 4°C. Both the supernatants (Acid-labile iron pool) and the pellets (Protein-bound (Heme) iron pool) were subjected to mineralization and analysis of the Fe content. The percentage of iron measured in the two fractions (mean ± SD of 4 experiments) is reported.

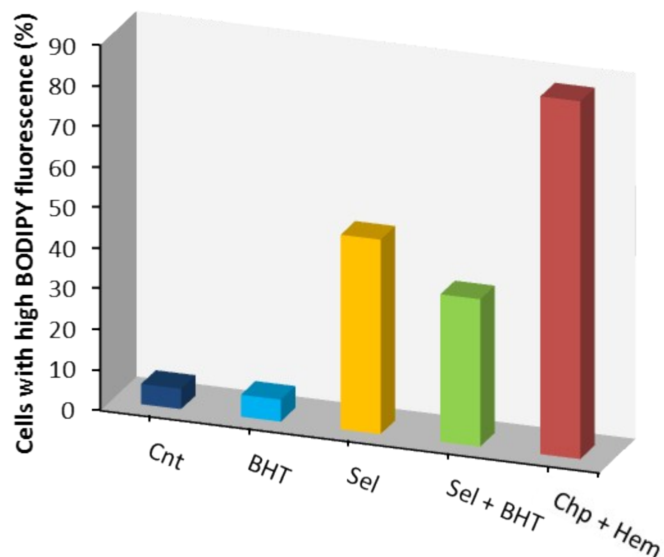

Figure S 5. FACS analysis of lipid peroxidation in HeLa cells treated with Sel +/- BHT using C11-BODIPY dye. HeLa cells (4.5 x 10^5) were treated with 15 μM Sel for 18 h with or without pre-incubation with 0.1 mM butylated hydroxytoluene (BHT) for 2 h and then subjected to the analysis of lipid peroxidation using C11-BODIPY dye as described in Experimental. Treatment of cells with 2.5 mM cumene hydroperoxide + 5 μM hemin (Chp + Hem) for 15 min is displayed as a positive control of lipid peroxidation induction.

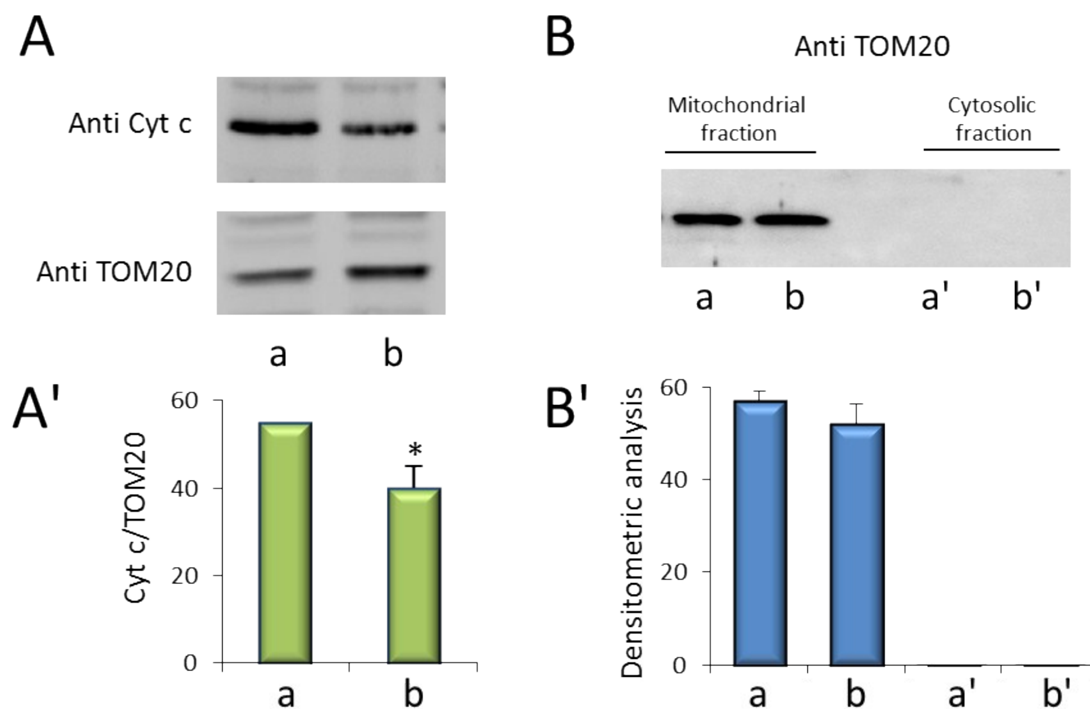

Figure S 6. Release of Cyt C after Sel treatment in HeLa cells
(A, A') Detection of Cyt c remaining in the mitochondrial fraction. Cells (4.5 x 10^5) were treated with 15 μM Sel for 18 h and then processed as reported in the Experimental section. **(A)** Aliquots of 10 μg protein of the mitochondrial fractions were subjected to SDS-PAGE (15%) followed by Western blot using a Cyt c monoclonal antibody. TOM20 content was determined as a loading control. **(A')** Relative amount of Cyt c in the mitochondrial fraction of control (a) or Sel-treated (b) HeLa cells showing a decrease of Cyt c in Sel-treated mitochondria. The graph reports the mean ± SD of 3 experiments (*$p<0.05$). **(B)** Estimation of the purity of the cytosolic cellular fraction (absence of mitochondrial residues) through the analysis of the amount of the mitochondrial marker TOM20. **(B')** Densitometric analysis of the Western blot bands reported in **B** utilizing the Nine-Alliance software. Control (**a, a'**); Sel-treated (**b, b'**).

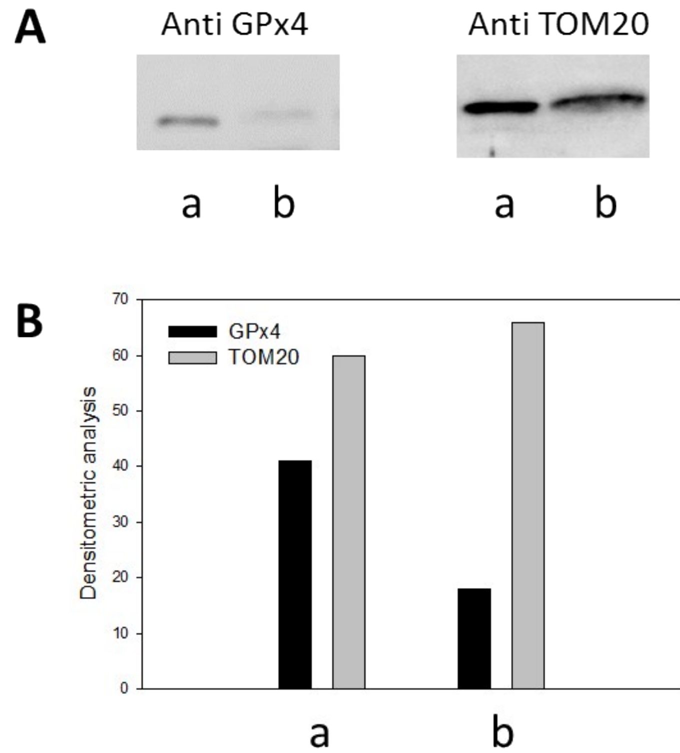

Figure S 7. Western blot analysis of GPx4 in mitochondria samples in the presence of 15 μM SeI. **(A)** Level of GPx4 decreases in mitochondria of HeLa cells treated with 15 μM SeI for 18 h. In the same, the detection of TOM20 was performed as loading control. **(B)** Densitometric analysis of the Western blot bands reported in **A** utilizing the ImageJ software. **a**: Cnt; **b**:15 μM SeI.