

Supplemental Data to Mandal et al.

System x_C^- and Thioredoxin Reductase 1 Cooperatively Rescue Glutathione Deficiency

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Figure S1. Stable Expression of xCT in γ -GCS^{-/-} Cells.

(A) As reported previously, intracellular GSH was not detectable in γ -GCS^{-/-} cells by HPLC analysis. *E14 2 α TG* wild-type embryonic stem cells were used as a positive control.

(B) Proliferation of γ -GCS^{-/-} cells was dependent on NAC (5 mM) or GSH (2.5 mM) supplementation.

(C) Northern blot analysis revealed strong hybridisation signals of the bicistronic 2.7 kb xCT-IRES-puromycin acetyltransferase mRNA in xCT-transfected (xCT-5 and xCT-7), but not in mock- and eGFP-transfected cells.

(D) Quantitative RT-PCR analysis detected xCT expression in clone xCT-5 and xCT-7 with crossing points at 21.54 and 23.02 cycles, respectively. xCT-5 had approximately 8-fold higher xCT expression levels than clone xCT-7 (compare Northern blot). In contrast, no xCT signals were detected even after 50 amplification cycles in control cell lines, indicating that xCT expression is virtually absent in these cells.

Figure S2. xCT-Expressing Cells were Equally Resistant to Pro-oxidants and Genotoxic Agents as GSH-Supplemented Cells.

(A-D) Cells were treated with various pro-oxidants and genotoxic agents as indicated. The number of viable cells was determined 48 h after treatment by trypan blue exclusion. Error bars indicate mean \pm SD.

Figure S3. xCT-Expressing Cells are Fully Resistant to Chemical Inhibition of γ -GCS.

(A) NAC-supplemented eGFP-transfected cells and non-supplemented xCT-transfected γ -GCS^{-/-} cells were treated with high concentrations of BSO for 72 h and the number of viable cells was determined by trypan blue exclusion. Error bars indicate mean \pm SD.

(B) Phase contrast microscopy of eGFP-transfected γ -GCS^{-/-} cells in the presence of NAC treated for 10 passages with 1 mM BSO.

Figure S4. Antioxidant Supplementation Fails to Rescue *Txnrd1*^{-/-} Cells from BSO-Induced Cell Death.

Cells were treated with BSO either in the presence or absence of antioxidant supplements for 24 h and then stained with Annexin V and PI and analyzed by flow cytometry. The tested antioxidants were able to rescue *Txnrd1*^{+/-} control cells (18.7% Annexin V-PI positive cells in case of BSO alone v/s 1.8% with NAC, 4.3% with Trolox[®] and 4.0% with 2-ME), but were ineffective in preventing BSO-induced cell death in *Txnrd1*^{-/-} cells (lower panel). The experiment was repeated with another *Txnrd1*^{-/-} cell line with similar results. Pooled data from two independent experiments is represented in percentage (mean \pm SD).

Figure S1

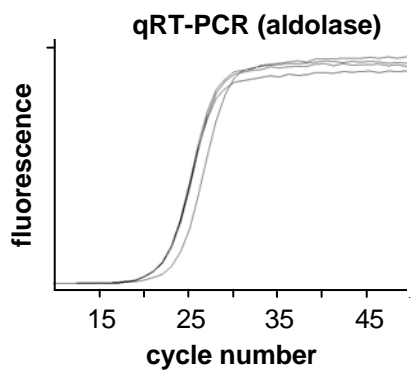
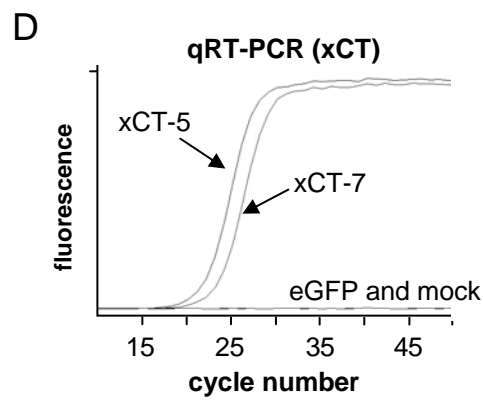
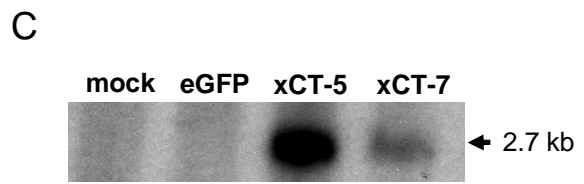
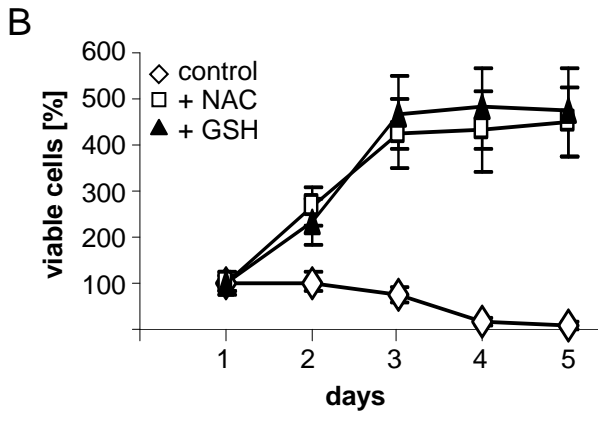
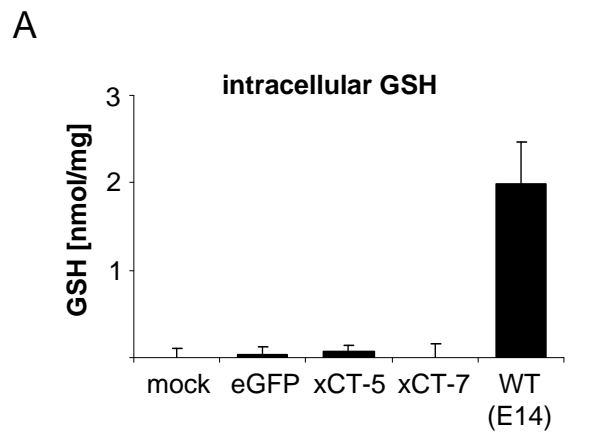
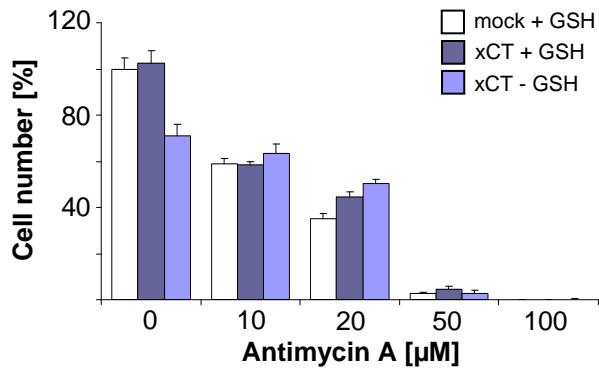
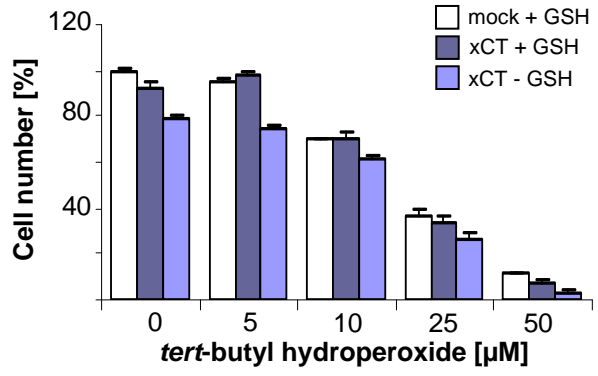


Figure S2

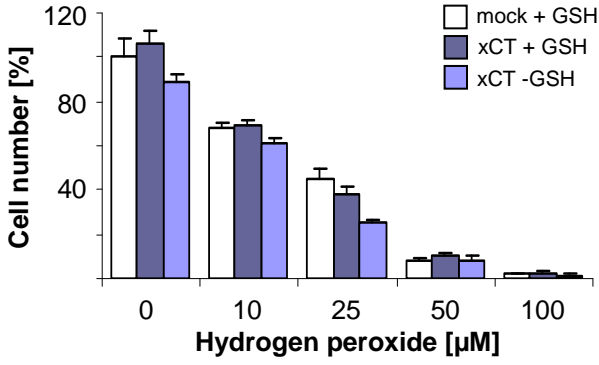
A



B



C



D

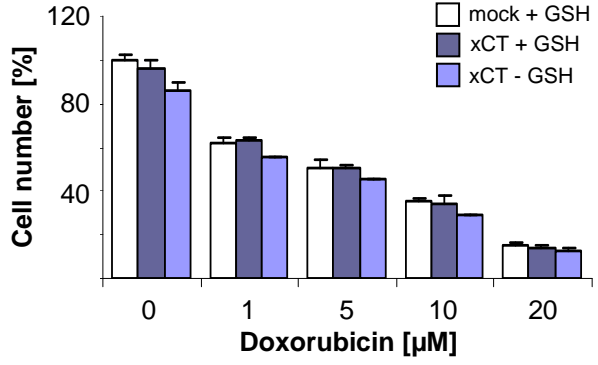
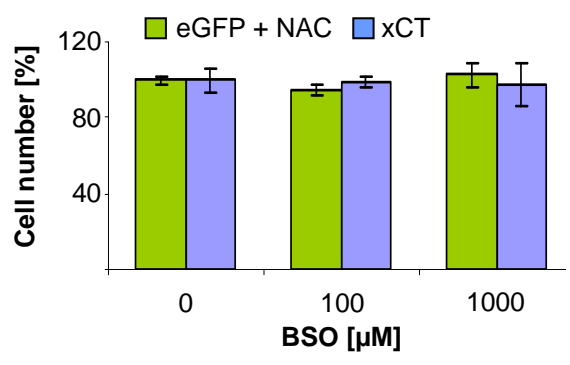


Figure S3

A



B

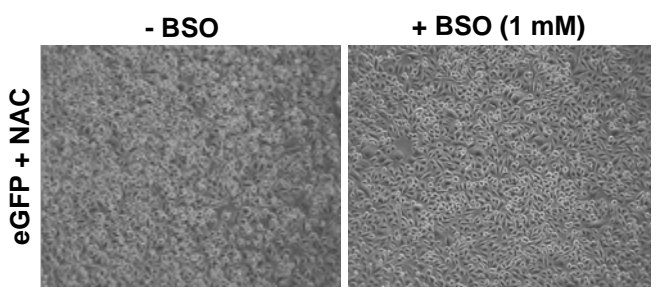


Figure S4

